

PATENT APPLICATION

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Docket No:

28341/6227.1NCP

### PATENT APPLICATION TRANSMITTAL UNDER 37 C.F.R. 1.53

Box Patent Application Assistant Commissioner for Patents Washington, D.C. 20231

Sir:

Transmitted herewith for filing is the patent application of

Inventor(s):

David E. Lowery, Troy E. Fuller and Michael J. Kennedy

Title:

ANTI-BACTERIAL VACCINE COMPOSITIONS

#### 1. Type of Application

$\boxtimes$	This i	s a	new	application	for	į
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 $\boxtimes$ 

utility patent.

design patent.

This is a continuation-in-part application of prior application no.

#### 2. Application Papers Enclosed

1 Title Page

Pages of Specification (excluding Claims, Abstract, Drawings & Sequence

Listing)

8 Page(s) of Claims

1 Page(s) of Abstract

0 Sheet(s) of Drawings (Figs. \_\_ to \_\_)

□ Formal

□ Informal

259 Page(s) of Sequence Listing

#### **CERTIFICATION UNDER 37 CFR 1.10**

I hereby certify that this Patent Application Transmittal and the documents referred to as enclosed therewith are being deposited with the United States Postal Service on **April 6, 2000**, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231 utilizing the "Express Mail Post Office to Addressee" service of the United States Postal Service under Mailing Label No. EM362733684US.

Richard Zimmermann

Other

3.	Declaration	or Oath	
		Enclosed	
		□ Execu	uted by (check all applicable boxes)
			Inventor(s)
			Legal representative of inventor(s) (37 CFR 1.42 or 1.43)
			Joint inventor or person showing a proprietary interest on behalf of inventor who refused to sign or cannot be reached
			☐ The petition required by 37 CFR 1.47 and the statement required by 37 CFR 1.47 are enclosed. See Item 5D below for fee.
	⊠	Not enclosed application on	- the undersigned attorney or agent is authorized to file this n behalf of the applicant(s). An executed declaration will follow.
4.	Additional	Papers Enclo	osed
		Preliminary A	mendment
		Information D	Disclosure Statement
		Declaration of	f Biological Deposit
	⋈		adable copy of sequence listing containing nucleotide and/or amino e ans statement under 37 C.F.R. §1.821
		Microfiche co	omputer program
		Verified stater	ment(s) claiming small entity status under 37 CFR 1.9 and 1.27
		Associate Pov	wer of Attorney
		Verified trans	slation of a non-English patent application
		An assignme	ent of the invention
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5.	Priority	<b>Applications</b>	Under	35	USC	1	1	9
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Certified copies of applications from which priority under 35 USC 119 is claimed are listed below and

- $\Box$  are attached.
- □ will follow.

COUNTRY	APPLICATION NO.	FILED	

- 6. Filing Fee Calculation (37 CFR 1.16)
  - A. 

    Utility Application

CLAIMS AS FILED - INCLUDING PRELIMINARY AMENDMENT (IF ANY)						
		SMALL ENTITY		OTHER THAN A SMALL ENTITY		
	NO. FILED	NO. EXTRA	RATE	FEE	RATE	FEE
BASIC FEE	Schrift (1965) A State (1975) Schrift (1965) A State (1975) Spanning (1975)			\$345.00	Chical Control	\$690.00
TOTAL	51 -20	= 31	X 9 =	\$	X 18 =	\$558.00
INDEP.	11 - 3	= 8	X 39 =	\$	X 78 =	\$624.00
☑ First Presentation of Multiple Dependent			+ 130 =	\$	+ 260 =	\$260.00
Filing Fee: \$				OR	\$2,132.00	

В.		Design Application (\$155.00/\$310.00)	Filing Fee:	\$
C.		Plant Application (\$240.00/\$480.00)	Filing Fee:	\$
D.	Other	Fees		
		Recording Assignment [Fee \$40.00 per assign	ment]	\$
		Petition fee for filing by other than all the inventors or person on behalf of the inventor where inventor to sign or cannot be reached [Fee \$130.00]	refused	\$
		Other		\$

Total Fees Enclosed \$2,132.00

$\boxtimes$	Enclosed check in the amount of:	\$ <u>2,132.00</u>
	Charge Deposit Account No. 13-2855 in the amount of: A copy of this Transmittal is enclosed.	\$
	Not enclosed	

#### 8. Deposit Account and Refund Authorization

The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 37 CFR 1.17 or under other applicable rules (except payment of issue fees), to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.

Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Please direct all future communications to Joseph A. Williams, Jr., at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN, MURRAY & BORUN 6300 Sears Tower 233 South Wacker Drive Chicago, Illinois 60606-6402 (312) 474-6300 (312) 474-0448 (Telefacsimile)

By:

Joseph A. Williams, Jr.

April 6, 2000

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Date of Deposit: April 6, 2000

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Commissioner for Patents, Washington, D.C.

Richard Zimmermann

## APPLICATION FOR UNITED STATES LETTERS PATENT

20231

## SPECIFICATION

#### TO ALL WHOM IT MAY CONCERN:

Be it known that we, David E. Lowery a citizen of the United States of America, residing at 1207 Woodland Drive, Portage, 49024 in the County of Kalamazoo and State of Michigan and Troy E. Fuller a citizen of the United States of America, residing at 111 Dreamfield Drive, Battle Creek, 49014, in the County of Calhoun and State of Michigan and Michael J. Kennedy a citizen of the United States of America, residing at 2364 Quincy Avenue, Portage, 49024, in the County of Kalamazoo and State of Michigan have invented a new and useful ANTI-BACTERIAL VACCINE COMPOSITIONS, of which the following is a specification.

New Patent Application for:

David E. Lowery, Troy E. Fuller, and

Michael J. Kennedy

For:

ANTI-BACTERIAL VACCINE COMPOSITIONS

Mailing Certification for:

**New Patent Application** 

Attorney Docket No:

28341/6227.1NCP

"EXPRESS MAIL" mailing label No. EM362733684US

Date of Deposit:

April 6, 2000

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Richard Zimmermann

#### Certificate of 37 C.F.R. §1.821 (f)

I hereby state that the content of the paper and computer readable copies of the Sequence Listing, submitted in accordance with 37 C.F.R. §1.821(c) and (e), respectively, are the same.

Joseph A. Williams, Jr.

# ANTI-BACTERIAL VACCINE COMPOSITIONS

#### FIELD OF THE INVENTION

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The present invention relates generally to the identification of genes responsible for virulence of *Pasteurella multocida* and *Actinobacillus pleuropneumoniae* bacteria, thereby allowing for production of novel attenuated mutant strains useful in vaccines and identification of new anti-bacterial agents that target the virulence genes and their products.

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#### BACKGROUND OF THE INVENTION

The family *Pasteurellaceae* encompasses several significant pathogens that infect a wide variety of animals. In addition to *P. multocida*, prominent members of the family include *Pasteurella haemolytica*, *Actinobacillus pleuropneumoniae* and *Haemophilus somnus*. *P. multocida* is a gram-negative, nonmotile coccobacillus which is found in the normal flora of many wild and domestic animals and is known to cause disease in numerous animal species worldwide [Biberstein, In M. Kilian, W. Frederickson, and E. L. Biberstein (ed.), *Haemophilus*, *Pasteurella*, *and Actinobacillus*. Academic Press, London, p. 61-73 (1981)]. The disease manifestations following infection include septicemias, bronchopneumonias, rhinitis, and wound infections [Reviewed in Shewen, *et al.*, *In* C. L. Gyles and C. O. Thoen (ed.), <u>Pathogenesis of Bacterial Infections in Animals</u>. Iowa State University Press, Ames, p. 216-225 (1993), incorporated herein by reference].

Infection by P. multocida generally results from invasion during periods

of stress, but transmission may also occur by aerosol or contact exposure, or via flea and tick vectors. In fowl, *P. multocida* infection gives rise to acute to peracute septicemia, particularly prevalent in domestic turkeys and wild waterfowl under stress conditions associated with overcrowding, laying, molting, or severe climatic change. In cattle, a similar hemorrhagic septicemia follows infection and manifests conditions including high fever and depression, generally followed by quick death. Transmission is most likely through aerosol contact, but infection can also arise during periods of significant climatic

change. In rabbits, infection gives rise to recurring purulent rhinitis, generally followed by conjunctivitis, otitis media, sinusitis, subcutaneous abscesses, and chronic

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bronchopneumonia. In severe infections, rabbit mortality arises from acute fibrinous bronchopneumonia, septicemia, or endotoxemia. Disease states normally arise during periods of stress. In pigs, common *P. multocida* disease states include atrophic rhinitis and bacterial pneumonia. Similar pneumonia conditions are also detected in dogs, cats, goats, and sheep. *P. multocida* is commonly detected in oral flora of many animals and is therefore a common contaminant in bite and scratch wounds.

P. multocida strains are normally designated by capsular serogroup and somatic serotype. Five capsular serogroups (A, B, D, E, and F) and 16 somatic serotypes are distinguished by expression of characteristic heat-stable antigens. Most strains are host specific and rarely infect more than one or two animals. The existence of different serotypes presents a problem for vaccination because traditional killed whole cell bacteria normally provide only serotype-specific protection. However, it has been demonstrated that natural infection with one serotype can lead to immunological protection against multiple serotypes [Shewen, et al., In C. L. Gyles and C. O. Thoen (Ed.), Pathogenesis of Bacterial Infections in Animals. Iowa State University Press, Ames, p. 216-225 (1993)] and cross protection can also be stimulated by using inactivated bacteria grown in vivo [Rimler, et al., Am J Vet Res. 42:2117-2121 (1981)]. One live spontaneous mutant P. multocida strain has been utilized as a vaccine and has been shown to stimulate a strong immune response [Davis, Poultry Digest. 20:430-434 (1987), Schlink, et al., Avian Dis. 31(1):13-21 (1987)]. This attenuated strain, however, has been shown to revert to a virulent state or cause mortality if the vaccine recipient is stressed [Davis, Poultry Digest. 20:430-434 (1987), Schlink, et al., Avian Dis. 31(1):13-21 (1987)].

Another member of the *Pasteurella* family, *A. pleuropneumoniae* exhibits strict host specificity for swine and is the causative agent of highly contagious porcine pleuropneumonia. Infection normally arises in intensive breeding conditions, and is believed to occur by a direct mode of transmission. The disease is often fatal and, as a result, leads to severe economic loss in the swine producing industry. *A. pleuropneumoniae* infection may be chronic or acute, and infection is characterized by a hemorrhagic, necrotic bronchopneumonia with accompanying fibrinous pleuritis. To date, bacterial virulence has been attributed to structural proteins, including serotype-specific capsular polysaccharides, lipopolysaccharides, and surface proteins, as well as

extracellular cytolytic toxins. Despite purification and, in some instances cloning, of these virulence factors, the exact role of these virulence factors in *A. pleuropneumoniae* infection is poorly understood.

Twelve serotypes of *A. pleuropneumoniae* have been identified based on antigenic differences in capsular polysaccharides and production of extracellular toxins. Serotypes 1, 5, and 7 are most relevant to *A. pleuropneumoniae* infection in the United States, while serotypes 1, 2, 5, 7, and 9 are predominant in Europe. There are at least three significant extracellular toxins of *A. pleuropneumoniae* that are members of the haemolysin family and are referred to as RTX toxins. RTX toxins are produced by many Gram negative bacteria, including *E. coli, Proteus vulgarisa*, and *Pasteurella haemolytica*, and the proteins generally share structural and functional characteristics. Toxins from the various serotypes differ, however, in host specificity, target cells, and biological activities.

The major *A. pleuropneumoniae* RTX toxins include ApxI, ApxII, and ApxIII. ApxI and ApxII have haemolytic activity, with ApxI being more potent. ApxIII shows no haemolytic activity, but is cytotoxic for alveolar macrophages and neutrophils. Most *A. pleuropneumoniae* serotypes produce two of these three toxins. For example, serotypes 1, 5, 9, and 11 express ApxI and ApxII, and serotypes 2, 3, 4, 6, and 8 express ApxII and ApxIII. Serotype 10, however, produces only ApxI, and serotypes 7 and 12 express only ApxII. Those *A. pleuropneumoniae* serotypes that produce both ApxI and ApxIII are the most virulent strains of the bacteria.

The Apx toxins were demonstrated to be virulence factors in murine models and swine infection using randomly mutated wild type bacteria [Tascon, et al., Mol. Microbiol. 14:207-216 (1994)]. Other A. pleuropneumoniae mutants have also been generated with targeted mutagenesis to inactivate the gene encoding the AopA outer membrane virulence protein [Mulks and Buysee, Gene 165:61-66 (1995)].

In attempts to produce vaccine compositions, traditional killed whole cell bacteria have provided only serotype-specific protection [MacInnes and Smart, *supra*], however, it has been demonstrated that natural infection with a highly virulent serotype can stimulate strong protective immunity against multiple serotypes [Nielsen, *Nord Vet Med. 31*:407-13 (1979), Nielsen, *Nord Vet Med. 36*:221-234 (1984), Nielsen, *Can J Vet* 

Res. 29:580-582 (1988), Nielsen, ACTA Vet Scand. 15:80-89 (1994)]. One defined live-attenuated vaccine strain producing an inactive form of the ApxII toxin has shown promise for cross protection in swine [Prideaux, et al., Infection & Immunity 67:1962-1966 (1999)], while other undefined live-attenuated mutants have also shown promise [Inzana, et al., Infect Immun. 61:1682-6, (1993), Paltineanu, et al., In International Pig Veterinary Society, 1992, p. 214, Utrera, et al., In International Pig Veterinary Society, 1992, p. 213].

Because of the problems associated with vaccine formulations comprising bacterial strains with undefined, spontaneous mutations, there exists a need in the art for rational construction of live attenuated bacterial strains for use in vaccines that will safely stimulate protective immunity against homologous and heterologous *P. multocida* and *A. pleuropneumoniae* serotypes. There further exists a need to identify attenuated bacterial strains and genes required for bacterial virulence, thereby facilitating development of methods to identify anti-bacterial agents.

#### SUMMARY OF THE INVENTION

In general, the present invention provides materials and methods for production and use of vaccine compositions comprising attenuated gram negative bacteria. In one aspect, vaccine compositions of the invention comprise attenuated species in the *Pasteurellaceae* family of bacteria, which is known in the art and described, in part, in Dewhirst, *et al.*, *J. Bacteriol. 174*:2002-2013 (1992), incorporated herein by reference in its entirety. Species in the family include, but are not limited to, *A. actinomycetemcomitans*, *A. capsulatus*, *A. equuli*, *A. lignieresii*, *A. pleuropneumoniae* (*H. pleuropneumoniae*), *A. seminis*, *A. suis* (*H. suis*), *A. ureae* (*p. ureae*), *A. capsulatus*, Bisgaard taxon 11, *H. aegyptius*, *H. aphrophilus*, *H. aphrophilus* (*H. parainfluenzae*), *H. ducreyi*, *H.* haemoglobinophilus, *H. haemolyticus*, *H. influenzae*, *H. paracuniculus*, *H. paraphrophilus*, *H. paraphrophilus*, *H. parasuis* type 5, *H. segnis*, *H. somnus*, *Haemophilus* minor group, *Haemophilus* taxon C, *P. aerogenes*, *P. anatis*, *P. avium* (*H. avium*), *P. canis*, *P. dagmatis*, *P. gallinarum*, *P. haemolytica*, *P. trehalosi* (*P. haemolytica* biotype T), *P. langaa*, *P. multocida*, *P. pneumotropica*, *P. stomatis*, *P. stomatis*, *P. stomatis*, *P. stomatis*, *P. pneumotropica*, *P. stomatis*, *P. st* 

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volantium (H. parainfluenzae), P. volantium, Pasteurella species A, Pasteurella species B, and Haemophilus paraphrohaemolyticus. Preferably, vaccine compositions comprise attenuated Pasteurella haemolytica, Actinobacillus pleuropneumoniae, Haemophilus somnus, or Pasteurella multocida bacteria. In a most preferred embodiment, vaccine compositions of the invention comprise attenuated Pasteurella multocida and A. plueropneumoniae bacterial strains.

One aspect of the invention provides gram negative bacterial organisms containing a functional mutation in a gene sequence represented by any one of SEQ ID NOS: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, and 164, or species homologs thereof, wherein the mutation inhibits or abolishes expression and/or biological activity of an encoded gene product (i.e., the polypeptide encoded by a gene); said functional mutation resulting in attenuated virulence of the bacterial strain. As understood in the art, species homologs include genes found in two or more different species which possess substantial polynucleotide sequence homology and possess the same, or similar, biological functions and/or properties. Preferably polynucleotide sequences which represent species homologs will hybridize under moderately stringent conditions, as described herein by example, and possess the same or similar biological activities and or properties. In another aspect, polynucleotides representing species homologs will share greater than about 60% sequence homology, greater than about 70% sequence homology, greater than about 80% sequence homology, greater than about 90% sequence homology or greater than about 95% sequence homology. Functional mutations that modulate (i.e., increase or decrease) expression and/or biological activity of a gene product include insertions or deletions in the protein coding region of the gene itself or in sequences responsible for, or involved in, control of gene expression. Deletion mutants include those wherein all or part of a specific gene sequence is deleted. In one aspect, the mutation results in deletion of at least about 10%, at least about 20%, at least about 30%, at least about 40% at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98%, or at least about 99% of said gene. In another aspect, the mutation results in an insertion in the gene, wherein the insertion causes decreased expression of a gene product encoded by the mutated gene and/or expression of an inactive gene product encoded by the mutated gene. Also contemplated are compositions, and preferably vaccine compositions, comprising mutated and attenuated gram negative bacterial organisms, optionally comprising a suitable adjuvant and/or a pharmaceutically acceptable diluent or carrier. In order for a modified strain to be effective in a vaccine formulation, the attenuation must be significant enough to prevent the pathogen from evoking severe clinical symptoms, but also insignificant enough to allow limited replication and growth of the bacteria in the host.

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The invention also provides polynucleotides encoding gene products that are required for virulence in gram negative bacteria. Polynucleotides of the invention include DNA, such as complementary DNA, genomic DNA including complementary or anti-sense DNA, and wholly or partially synthesized DNA; RNA, including sense and antisense strands; and peptide nucleic acids as described, for example in Corey, TIBTECH 15:224-229 (1997). Virulence gene polynucleotides of the invention include those set forth in SEO ID NOs:1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, and 164, or species homologs thereof, polynucleotides encoding a virulence gene product encoded by a polynucleotide of SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, and 164, or a species homolog thereof, and polynucleotide that hybridize, under moderately to highly stringent conditions, to the noncoding strand (or complement) of any one of the polynucleotides set out in SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, and 164, species homologs thereof. The invention therefore comprehends gene sequences from Pasteurellaceae set out in SEQ ID NOs: 1,

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3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, and 164, as well as related gene sequences from other gram negative bacterial organisms, including naturally occurring (*i.e.*, species homologs) and artificially induced variants thereof. The invention also comprehends polynucleotides which encode polypeptides deduced from any one of the polynucleotides set out in SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, and 164, and species homologs thereof. Knowledge of the sequence of a polynucleotide of the invention makes readily available every possible fragment of that polynucleotide. The invention therefore provides fragments of a polynucleotide of the invention.

The invention further embraces expression constructs comprising polynucleotides of the invention. Host cells transformed, transfected or electroporated with a polynucleotide of the invention are also contemplated. The invention provides

methods to produce a polypeptide encoded by a polynucleotide of the invention comprising the steps of growing a host cell of the invention under conditions that permit, and preferably promote, expression of a gene product encoded by the polynucleotide, and

isolating the gene product from the host cell or the medium of its growth.

Identification of polynucleotides of the invention makes available the encoded polypeptides. Polypeptides of the invention include full length and fragment, or truncated, proteins; variants thereof; fusion, or chimeric proteins; and analogs, including those wherein conservative amino acid substitutions have been introduced into wild-type polypeptides. Antibodies that specifically recognize polypeptides of the invention are also provided, and include monoclonal and polyclonal antibodies, single chain antibodies, chimeric antibodies, humanized antibodies, human antibodies, and complementary determining region (CDR)-grafted antibodies, as well as compounds that include CDR sequences which specifically recognize a polypeptide of the invention. The

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invention also provides anti-idiotype antibodies immunospecific for antibodies of the invention.

According to another aspect of the invention, methods are provided for identifying novel anti-bacterial agents that modulate the function of gram negative bacteria virulence genes or gene products. Methods of the invention include screening potential agents for the ability to interfere with expression of virulence gene products encoded by the DNA sequences set forth in any one of SEQ ID NOS: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, and 164, or species homologs thereof, or screening potential agents for the ability to interfere with biological function of a bacterial gene product encoded in whole or in part by a DNA sequence set forth in any one of SEQ ID NOS: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, and 164, species homologs thereof, or the complementary strand thereof, followed by identifying agents that provide positive results in such screening assays. In particular, agents that interfere with the expression of virulence gene products include anti-sense polynucleotides and ribozymes that are complementary to the virulence gene sequences. The invention further embraces methods to modulate transcription of gene products of the invention through use of oligonucleotide-directed triplet helix formation.

Agents that interfere with the function of virulence gene products include variants of virulence gene products, binding partners of the virulence gene products and variants of such binding partners, and enzyme inhibitors (where the product is an enzyme).

Novel anti-bacterial agents identified by the methods described herein are provided, as well as methods for treating a subject suffering from infection with gram negative bacteria involving administration of such novel anti-bacterial agents in an amount effective to reduce bacterial presence.

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Numerous additional aspects and advantages of the invention will become apparent to those skilled in the art upon consideration of the following detailed description of the invention which describes presently prepared embodiments thereof.

#### DETAILED DESCRIPTION OF THE INVENTION

"Virulence genes," as used herein, are genes whose function or products are required for successful establishment and/or maintenance of bacterial infection in a host animal. Thus, virulence genes and/or the proteins encoded thereby are involved in pathogenesis in the host organism, but may not be necessary for growth.

"Signature-tagged mutagenesis (STM)," as used herein, is a method generally described in International Patent Publication No. WO 96/17951, incorporated herein by reference, and includes, for example, a method for identifying bacterial genes required for virulence in a murine model of bacteremia. In this method, bacterial strains that each have a random mutation in the genome are produced using transposon integration; each insertional mutation carries a different DNA signature tag which allows mutants to be differentiated from each other. The tags comprise 40 bp variable central regions flanked by invariant "arms" of 20 bp which allow the central portions to be co-amplified by polymerase chain reaction (PCR). Tagged mutant strains are assembled in microtiter dishes, then combined to form the "inoculum pool" for infection studies. At an appropriate time after inoculation, bacteria are isolated from the animal and pooled to form the "recovered pool." The tags in the recovered pool and the tags in the inoculum pool are separately amplified, labeled, and then used to probe filters arrayed with all of the different tags representing the mutants in the inoculum. Mutant strains with attenuated virulence are those which cannot be recovered from the infected animal, i.e., strains with tags that give hybridization signals when probed with tags from the inoculum pool but not when probed with tags from the recovered pool. In a variation of this method, non-radioactive detection methods such as chemiluminescence can be used

Signature-tagged mutagenesis allows a large number of insertional mutant strains to be screened simultaneously in a single animal for loss of virulence. Screening nineteen pools of mutant *P. multocida* strains resulted in the identification of more than 60 strains with reduced virulence, many of which were confirmed to be attenuated in

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virulence by subsequent determination of an approximate  $LD_{50}$  for the individual mutants. Screening of A. pleuropneumoniae mutants resulted in identification of more than 100 strains having mutations in 35 different genes. Of these, mutations in 22 genes results in significantly attenuated A. pleuropneumoniae strains. The nucleotide sequence of the open reading frame disrupted by the transposon insertion was determined by sequencing both strands and an encoded amino acid sequence was deduced. Novelty of both the polynucleotide and amino acid sequences was determined by comparison of the sequences with DNA and protein database sequences.

The identification of bacterial, and more particularly *P. multocida* and *A. pleuropneumoniae* virulence genes provides for microorganisms exhibiting reduced virulence (*i.e.*, attenuated strains), which are useful in vaccines. Such microorganisms include *Pasteurellaceae* mutants containing at least one functional mutation inactivating a gene represented by any one of SEQ ID NOS: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, and 164. The worker of ordinary skill in the art will realize that a "functional mutation" may occur in protein coding regions of a gene of the invention, as well as in regulatory regions that modulate transcription of the virulence gene RNA.

The worker of ordinary skill will also appreciate that attenuated *P. multocida* and *A. pleuropneumoniae* strains of the invention include those bearing more than one functional mutation. More than one mutation may result in additive or synergistic degrees of attenuation. Multiple mutations can be prepared by design or may fortuitously arise from a deletion event originally intended to introduce a single mutation. An example of an attenuated strain with multiple deletions is a *Salmonella typhimurium* strain wherein the *cya* and *crp* genes are functionally deleted. This mutant *S. typhimurium* strain has shown promise as a live vaccine.

Identification of virulence genes in *P. multocida* and *A. pleuropneumoniae* can provide information regarding similar genes, *i.e.*, species homologs, in other pathogenic species. As an example, identification of the *aroA* gene led to identification of conserved genes in a diverse number of pathogens, including *P. haemolytica*,

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Aeromonas hydrophila, Aeromonas salmonicida, Salmonella typhimurium, Salmonella enteritidis, Salmonella dublin, Salmonella gallanerum, Bordella pertussis, Yersinia entericolitica, Neisseria gonorrhoeae, and Bacillus anthracis. In many of these species, attenuated bacterial strains bearing mutations in the aroA gene have proven to be effective in vaccine formulations. Using the virulence genes sequences identified in P. multocida, similar or homologous genes can be identified in other organisms, particularly within the Pasteurella family, as well as A. pleuropneumoniae and Haemophilus somnus. Likewise, identification of A. pleuropneumoniae virulence genes can permit identification of related genes in other organisms. Southern hybridization using the P. multocida and A. pleuropneumoniae genes as probes can identify these related genes in chromosomal libraries derived from other organisms. Alternatively, PCR can be equally effective in gene identification across species boundaries. As still another alternative, complementation of, for example, a P. multocida mutant with a chromosomal library from other species can also be used to identify genes having the same or related virulence activity. Identification of related virulence genes can therefore lead to production of an attenuated strain of the other organism which can be useful as still another vaccine formulation. Examples of P. multocida genes that have been demonstrated to exist in other species (e.g. P. haemolytica, A. pleuropneumoniae and H. somnus) include genes exbB, atpG, and pnp

Attenuated *P. multocida* strains identified using STM are insertional mutants wherein a virulence gene has been rendered non-functional through insertion of transposon sequences in either the open reading frame or regulatory DNA sequences. In one aspect, therefore, the attenuated *P. multocida* strains, as well as other gram-negative mutant bacterial strains of the invention can bear one or more mutations which result in an insertion in the gene, with the insertion causing decreased expression of a gene product encoded by the mutated gene and/or expression of an inactive gene product encoded by the mutated gene. These insertional mutants still contain all of the genetic information required for bacterial virulence and can possibly revert to a pathogenic state by deletion of the inserted transposon. Therefore, in preparing a vaccine formulation, it is desirable to take the information gleaned from the attenuated strain and create a deletion mutant strain wherein some, most, or all of the virulence gene sequence is

removed, thereby precluding the possibility that the bacteria will revert to a virulent state. The attenuated *P. multocida* strains, as well as other gram-negative mutant bacterial strains of the invention therefore include those bearing one or more mutation which results in deletion of at least about 10%, at least about 20%, at least about 30%, at least about 30%, at least about 40% at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98%, or at least about 99% of the virulence gene.

The vaccine properties of an attenuated insertional mutant identified using STM are expected to be the same or similar to those of a bacteria bearing a deletion in the same gene. However, it is possible that an insertion mutation may exert "polar" effects on adjoining gene sequences, and as a result, the insertion mutant may possess characteristic distinct from a mutant strain with a deletion in the same gene sequence. Deletion mutants can be constructed using any of a number of techniques well known and routinely practiced in the art.

In one example, a strategy using counterselectable markers can be employed which has commonly been utilized to delete genes in many bacteria. For a review, see, for example, Reyrat, et al., Infection and Immunity 66:4011-4017 (1998), incorporated herein by reference. In this technique, a double selection strategy is often employed wherein a plasmid is constructed encoding both a selectable and counterselectable marker, with flanking DNA sequences derived from both sides of the desired deletion. The selectable marker is used to select for bacteria in which the plasmid has integrated into the genome in the appropriate location and manner. The counterselecteable marker is used to select for the very small percentage of bacteria that have spontaneously eliminated the integrated plasmid. A fraction of these bacteria will then contain only the desired deletion with no other foreign DNA present. The key to the use of this technique is the availability of a suitable counterselectable marker.

In another technique, the *cre-lox* system is used for site specific recombination of DNA. The system consists of 34 base pair *lox* sequences that are recognized by the bacterial *cre* recombinase gene. If the *lox* sites are present in the DNA in an appropriate orientation, DNA flanked by the *lox* sites will be excised by the *cre* recombinase, resulting in the deletion of all sequences except for one remaining copy of

the *lox* sequence. Using standard recombination techniques, it is possible to delete the targeted gene of interest in the *P. multocida* or *A. pleuropneumoniae* genome and to replace it with a selectable marker (*e.g.*, a gene coding for kanamycin resistance) that is flanked by the *lox* sites. Transient expression (by electroporation of a suicide plasmid containing the *cre* gene under control of a promoter that functions in *P. multocida* or *A. pleuropneumoniae*) of the *cre* recombinase should result in efficient elimination of the *lox* flanked marker. This process would result in a mutant containing the desired deletion mutation and one copy of the *lox* sequences.

In another approach, it is possible to directly replace a desired deleted sequence in the *P. multocida* or *A. pleuropneumoniae* genome with a marker gene, such as green fluorescent protein (GFP), β-galactosidase, or luciferase. In this technique, DNA segments flanking a desired deletion are prepared by PCR and cloned into a suicide (non-replicating) vector for *P. multocida* or *A. pleuropneumoniae*. An expression cassette, containing a promoter active in *P. multocida* or *A. pleuropneumoniae* and the appropriate marker gene, is cloned between the flanking sequences. The plasmid is introduced into wild-type *P. multocida* or *A. pleuropneumoniae*. Bacteria that incorporate and express the marker gene (probably at a very low frequency) are isolated and examined for the appropriate recombination event (*i.e.*, replacement of the wild type gene with the marker gene).

The reduced virulence of these organisms and their immunogenicity may be confirmed by administration to a subject animal. While it is possible for an avirulent microorganism of the invention to be administered alone, one or more of such mutant microorganisms are preferably administered in a vaccine composition containing suitable adjuvant(s) and pharmaceutically acceptable diluent(s) or carrier(s). The carrier(s) must be "acceptable" in the sense of being compatible with the avirulent microorganism of the invention and not deleterious to the subject to be immunized. Typically, the carriers will be water or saline which will be sterile and pyrogen free. The subject to be immunized is a subject needing protection from a disease caused by a virulent form of *P. multocida*, *A. pleuropneumoniae*, or other pathogenic microorganisms.

It will be appreciated that the vaccine of the invention may be useful in the fields of human medicine and veterinary medicine. Thus, the subject to be

immunized may be a human or other animal, for example, farm animals including cows, sheep, pigs, horses, goats and poultry (*e.g.*, chickens, turkeys, ducks and geese) companion animals such as dogs and cats; exotic and/or zoo animals; and laboratory animals including mice, rats, rabbits, guinea pigs, and hamsters.

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The invention also provides polypeptides and corresponding polynucleotides required for P. multocida or A. pleuropneumoniae virulence. The invention includes both naturally occurring and non-naturally occurring polynucleotides Naturally occurring virulence products include and polypeptide products thereof. distinct gene and polypeptide species as well as corresponding species homologs expressed in organisms other than P. multocida or A. pleuropneumoniae strains. Non-naturally occurring virulence products include variants of the naturally occurring products such as analogs and virulence products which include covalent modifications. In a preferred embodiment, the invention provides virulence polynucleotides comprising the sequences set forth in SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, and 164, and species homologs thereof, and polypeptides having amino acids sequences encoded by the polynucleotides.

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The present invention provides novel purified and isolated *P. multocida* and *A. pleuropneumoniae* polynucleotides (e.g., DNA sequences and RNA transcripts, both sense and complementary antisense strands) encoding the bacterial virulence gene products. DNA sequences of the invention include genomic and cDNA sequences as well as wholly or partially chemically synthesized DNA sequences. Genomic DNA of the invention comprises the protein coding region for a polypeptide of the invention and includes variants that may be found in other bacterial strains of the same species. "Synthesized," as used herein and is understood in the art, refers to purely chemical, as opposed to enzymatic, methods for producing polynucleotides. "Wholly" synthesized DNA sequences are therefore produced entirely by chemical means, and "partially" synthesized DNAs embrace those wherein only portions of the resulting DNA were produced by chemical means. Preferred DNA sequences encoding *P. multocida* 

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virulence gene products are set out in SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, and 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, and 164, and species homologs thereof. Preferred A. pleuropneumoniae DNA sequences encoding virulence gene products are set out in SEQ ID NOs: 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, and 164, and species homologs thereof. The worker of skill in the art will readily appreciate that the preferred DNA of the invention comprises a double stranded molecule, for example, molecules having the sequences set forth in SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, and 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, and 164, and species homologs thereof, along with the complementary molecule (the "noncoding strand" or "complement") having a sequence deducible from the sequence of SEQ ID NO: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, and 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, and 164, according to Watson-Crick base pairing rules for DNA. Also preferred are polynucleotides encoding the gene products encoded by any one of the polynucleotides set out in SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, and 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, and 164, and species homologs thereof. The invention further embraces species, preferably bacterial, homologs of the P. multocida and A. pleuropneumoniae DNA.

The polynucleotide sequence information provided by the invention makes possible the identification and isolation of polynucleotides encoding related bacterial virulence molecules by well known techniques including Southern and/or Northern hybridization, and polymerase chain reaction (PCR). Examples of related polynucleotides include polynucleotides encoding polypeptides homologous to a

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virulence gene product encoded by any one of the polynucleotides set out in SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, and 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, and 164, and species homologs thereof, and structurally related polypeptides sharing one or more biological and/or physical properties of a virulence gene product of the invention.

The invention also embraces DNA sequences encoding bacterial gene products which hybridize under moderately to highly stringent conditions to the non-coding strand, or complement, of any one of the polynucleotides set out in SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, and 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, and 164, and species homologs thereof. DNA sequences encoding virulence polypeptides which would hybridize thereto but for the degeneracy of the genetic code are contemplated by the invention. Exemplary high stringency conditions include a final wash in buffer comprising 0.2X SSC/0.1% SDS, at 65°C to 75°C, while exemplary moderate stringency conditions include a final wash in buffer comprising 2X SSC/0.1% SDS, at 35°C to 45°C. It is understood in the art that conditions of equivalent stringency can be achieved through variation of temperature and buffer, or salt concentration as described in Ausubel, et al. (Eds.), Protocols in Molecular Biology, John Wiley & Sons (1994), pp. 6.0.3 to 6.4.10. Modifications in hybridization conditions can be empirically determined or precisely calculated based on the length and the percentage of guanosine/cytosine (GC) base pairing of the probe. The hybridization conditions can be calculated as described in Sambrook, et al., (Eds.), Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York (1989), pp. 9.47 to 9.51.

Autonomously replicating recombinant expression constructions such as plasmid and viral DNA vectors incorporating virulence gene sequences are also provided. Expression constructs wherein virulence polypeptide-encoding polynucleotides are operatively linked to an endogenous or exogenous expression control DNA sequence and

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a transcription terminator are also provided. The virulence genes may be cloned by PCR, using *P. multocida* genomic DNA as the template. For ease of inserting the gene into expression vectors, PCR primers are chosen so that the PCR-amplified gene has a restriction enzyme site at the 5' end preceding the initiation codon ATG, and a restriction enzyme site at the 3' end after the termination codon TAG, TGA or TAA. If desirable, the codons in the gene are changed, without changing the amino acids, according to *E. coli* codon preference described by Grosjean and Fiers, *Gene*, 18:199-209 (1982), and Konigsberg and Godson, *Proc. Natl. Acad. Sci. (USA)*, 80:687-691 (1983). Optimization of codon usage may lead to an increase in the expression of the gene product when produced in *E. coli*. If the gene product is to be produced extracellularly, either in the periplasm of *E. coli* or other bacteria, or into the cell culture medium, the gene is cloned without its initiation codon and placed into an expression vector behind a signal sequence.

According to another aspect of the invention, host cells are provided, including procaryotic and eukaryotic cells, either stably or transiently transformed, transfected, or electroporated with polynucleotide sequences of the invention in a manner which permits expression of virulence polypeptides of the invention. Expression systems of the invention include bacterial, yeast, fungal, viral, invertebrate, and mammalian cells Host cells of the invention are a valuable source of immunogen for development of antibodies specifically immunoreactive with the virulence gene product. Host cells of the invention are conspicuously useful in methods for large scale production of virulence polypeptides wherein the cells are grown in a suitable culture medium and the desired polypeptide products are isolated from the cells or from the medium in which the cells are grown by, for example, immunoaffinity purification or any of the multitude of purification techniques well known and routinely practiced in the art. Any suitable host cell may be used for expression of the gene product, such as E. coli, other bacteria, including P. multocida, Bacillus and S. aureus, yeast, including Pichia pastoris and Saccharomyces cerevisiae, insect cells, or mammalian cells, including CHO cells, utilizing suitable vectors known in the art. Proteins may be produced directly or fused to a peptide or polypeptide, and either intracellularly or extracellularly by secretion into the periplasmic space of a bacterial cell or into the cell culture medium. Secretion of a protein requires a signal peptide (also known as pre-sequence); a number of signal sequences from prokaryotes and eukaryotes are known to function for the secretion of recombinant proteins. During the protein secretion process, the signal peptide is removed by signal peptidase to yield the mature protein.

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To simplify the protein purification process, a purification tag may be added either at the 5' or 3' end of the gene coding sequence. Commonly used purification tags include a stretch of six histidine residues (U.S. Patent Nos. 5,284,933 and 5,310,663), a streptavidin-affinity tag described by Schmidt and Skerra, *Protein Engineering*, 6:109-122 (1993), a FLAG peptide [Hopp *et al.*, *Biotechnology*, 6:1205-1210 (1988)], glutathione S-transferase [Smith and Johnson, *Gene*, 67:31-40 (1988)], and thioredoxin [LaVallie *et al.*, *Bio/Technology*, 11:187-193 (1993)]. To remove these peptide or polypeptides, a proteolytic cleavage recognition site may be inserted at the fusion junction. Commonly used proteases are factor Xa, thrombin, and enterokinase.

The invention also provides purified and isolated P. multocida and A.

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pleuropneumoniae virulence polypeptides encoded by a polynucleotide of the invention. Presently preferred are polypeptides comprising the amino acid sequences encoded by any one of the polynucleotides set out in SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, and 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, and 164, and species homologs thereof. The invention embraces virulence polypeptides encoded by a DNA selected from the group consisting of: a) the DNA sequence set out in any one of SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, and 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, and 164, and species homologs thereof; b) DNA molecules encoding P. multocida or A. pleuropneumoniae polypeptides encoded by any one of SEO ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, and 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142,

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144, 146, 148, 150, 152, 154, 156, 158, 160, and 164, and species homologs thereof; and

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c) a DNA molecule, encoding a virulence gene product, that hybridizes under moderately stringent conditions to the DNA of (a) or (b).

The invention also embraces polypeptides, i.e., species homologs and orthologs, that have at least about 99%, at least about 95%, at least about 90%, at least about 85%, at least about 80%, at least about 75%, at least about 70%, at least about 65%, at least about 60%, at least about 55%, and at least about 50% identity and/or homology to the preferred polypeptides of the invention. Percent amino acid sequence "identity" with respect to the preferred polypeptides of the invention is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the residues in the virulence gene product sequence after aligning both sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Percent sequence "homology" with respect to the preferred polypeptides of the invention is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the residues in one of the virulence polypeptide sequences after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and also considering any conservative substitutions as part of the sequence identity. Conservative substitutions can be defined as set out in Tables A and В.

Table A
Conservative Substitutions I

	SIDE CHAIN O	CHARACTERISTIC	AMINO ACID
25	Aliphatic	Non-polar	G A P
	•	•	ILV
		Polar - uncharged	C S T M
			N Q
		Polar - charged	DE
30			KR
	Aromatic		HFWY
	Other		NQDE

Polypeptides of the invention may be isolated from natural bacterial cell sources or may be chemically synthesized, but are preferably produced by recombinant

procedures involving host cells of the invention. Virulence gene products of the invention may be full length polypeptides, biologically active fragments, or variants thereof which retain specific biological or immunological activity. Variants may comprise virulence polypeptide analogs wherein one or more of the specified (*i.e.*, naturally encoded) amino acids is deleted or replaced or wherein one or more non-specified amino acids are added: (1) without loss of one or more of the biological activities or immunological characteristics specific for the virulence gene product; or (2) with specific disablement of a particular biological activity of the virulence gene product. Deletion variants contemplated also include fragments lacking portions of the polypeptide not essential for biological activity, and insertion variants include fusion polypeptides in which the wild-type polypeptide or fragment thereof have been fused to another polypeptide.

Variant virulence polypeptides include those wherein conservative substitutions have been introduced by modification of polynucleotides encoding polypeptides of the invention. Conservative substitutions are recognized in the art to classify amino acids according to their related physical properties and can be defined as set out in Table A (from WO 97/09433, page 10, published March 13, 1997 (PCT/GB96/02197, filed 9/6/96). Alternatively, conservative amino acids can be grouped as defined in Lehninger, [Biochemistry, Second Edition; Worth Publishers, Inc. NY:NY (1975), pp.71-77] as set out in Table B.

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Table B
Conservative Substitutions II

5	SIDE CHAIN CHARACTERISTIC	AMINO ACID
	Non-polar (hydrophobic)	
	A. Aliphatic:	ALIVP
	B. Aromatic:	FW
10	C. Sulfur-containing:	M
	D. Borderline:	G
	Uncharged-polar	
	A. Hydroxyl:	S T Y
	B. Amides:	ΝQ
15	C. Sulfhydryl:	C
	D. Borderline:	G
	Positively Charged (Basic):	KRH
	Negatively Charged (Acidic):	
	DE	

Variant virulence products of the invention include mature virulence gene products, *i.e.*, wherein leader or signal sequences are removed, having additional amino terminal residues. Virulence gene products having an additional methionine residue at position -1 are contemplated, as are virulence products having additional methionine and lysine residues at positions -2 and -1. Variants of these types are particularly useful for recombinant protein production in bacterial cell types. Variants of the invention also nclude gene products wherein amino terminal sequences derived from other proteins have been introduced, as well as variants comprising amino terminal sequences that are not found in naturally occurring proteins.

The invention also embraces variant polypeptides having additional amino acid residues which result from use of specific expression systems. For example, use of commercially available vectors that express a desired polypeptide as a fusion protein with glutathione-S-transferase (GST) provide the desired polypeptide having an additional glycine residue at position -1 following cleavage of the GST component from the desired polypeptide. Variants which result from expression using other vector systems are also contemplated.

Also comprehended by the present invention are antibodies (e.g., monoclonal and polyclonal antibodies, single chain antibodies, chimeric antibodies,

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humanized, human, and CDR-grafted antibodies, including compounds which include CDR sequences which specifically recognize a polypeptide of the invention) and other binding proteins specific for virulence gene products or fragments thereof. The term "specific for" indicates that the variable regions of the antibodies of the invention recognize and bind a virulence polypeptide exclusively (i.e., are able to distinguish a single virulence polypeptides from related virulence polypeptides despite sequence identity, homology, or similarity found in the family of polypeptides), but may also interact with other proteins (for example, S. aureus protein A or other antibodies in ELISA techniques) through interactions with sequences outside the variable region of the antibodies, and in particular, in the constant region of the molecule. Screening assays to determine binding specificity of an antibody of the invention are well known and routinely practiced in the art. For a comprehensive discussion of such assays, see Harlow et al. (Eds), Antibodies A Laboratory Manual; Cold Spring Harbor Laboratory; Cold Spring Harbor, NY (1988), Chapter 6. Antibodies that recognize and bind fragments of the virulence polypeptides of the invention are also contemplated, provided that the antibodies are first and foremost specific for, as defined above, a virulence polypeptide of the invention from which the fragment was derived.

The DNA and amino acid sequence information provided by the present invention also makes possible the systematic analysis of the structure and function of the virulence genes and their encoded gene products. Knowledge of a polynucleotide encoding a virulence gene product of the invention also makes available anti-sense polynucleotides which recognize and hybridize to polynucleotides encoding a virulence polypeptide of the invention. Full length and fragment anti-sense polynucleotides are provided. The worker of ordinary skill will appreciate that fragment anti-sense molecules of the invention include (i) those which specifically recognize and hybridize to a specific RNA (as determined by sequence comparison of DNA encoding a virulence polypeptide of the invention to DNA encoding other known molecules) as well as (ii) those which recognize and hybridize to RNA encoding variants of the family of virulence proteins. Antisense polynucleotides that hybridize to RNA encoding other members of the virulence family of proteins are also identifiable through sequence comparison to identify characteristic, or signature, sequences for the family of molecules.

The invention further contemplates methods to modulate gene expression through use of ribozymes. For a review, see Gibson and Shillitoe, *Mol. Biotech.* 7:125-137 (1997). Ribozyme technology can be utilized to inhibit translation of mRNA in a sequence specific manner through (i) the hybridization of a complementary RNA to a target mRNA and (ii) cleavage of the hybridized mRNA through nuclease activity inherent to the complementary strand. Ribozymes can be identified by empirical methods but more preferably are specifically designed based on accessible sites on the target mRNA [Bramlage, *et al.*, *Trends in Biotech* 16:434-438 (1998)]. Delivery of ribozymes to target cells can be accomplished using either exogenous or endogenous delivery techniques well known and routinely practiced in the art. Exogenous delivery methods can include use of targeting liposomes or direct local injection. Endogenous methods include use of viral vectors and non-viral plasmids.

Ribozymes can specifically modulate expression of virulence genes when designed to be complementary to regions unique to a polynucleotide encoding a virulence gene product. "Specifically modulate" therefore is intended to mean that ribozymes of the invention recognizes only a single polynucleotide. Similarly, ribozymes can be designed to modulate expression of all or some of a family of proteins. Ribozymes of this type are designed to recognize polynucleotide sequences conserved in all or some of the polynucleotides which encode the family of proteins.

The invention further embraces methods to modulate transcription of a virulence gene of the invention through use of oligonucleotide-directed triplet helix formation. For a review, see Lavrovsky, et al., Biochem. Mol. Med. 62:11-22 (1997). Triplet helix formation is accomplished using sequence specific oligonucleotides which hybridize to double stranded DNA in the major groove as defined in the Watson-Crick model. Hybridization of a sequence specific oligonucleotide can thereafter modulate activity of DNA-binding proteins, including, for example, transcription factors and polymerases. Preferred target sequences for hybridization include transcriptional regulatory regions that modulate virulence gene product expression. Oligonucleotides which are capable of triplet helix formation are also useful for site-specific covalent modification of target DNA sequences. Oligonucleotides useful for covalent

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modification are coupled to various DNA damaging agents as described in Lavrovsky, et al. [supra].

The identification of P. multocida and A. pleuropneumoniae virulence genes renders the genes and gene products useful in methods for identifying anti-bacterial agents. Such methods include assaying potential agents for the ability to interfere with expression of virulence gene products represented by the DNA sequences set forth in any one of SEQ ID NOS: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, and 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, and 164, and species homologs thereof (i.e., the genes represented by DNA sequences of SEQ ID NOS: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, and 120, 122, 124, 126, 128, 130, and 164, encode the virulence gene product, or the DNA sequences of SEQ ID NOS: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70,72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, and 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, and 164, are adjacent the gene encoding the virulence gene product, or are involved in regulation of expression of the virulence gene product), or assaying potential agents for the ability to interfere with the function of a bacterial gene product encoded in whole or in part by a DNA sequence set forth in any one of SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, and 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, and 164, species homologs thereof, or the complementary strand thereof, followed by identifying agents that are positive in such assays. Polynucleotides and polypeptides useful in these assays include not only the genes and encoded polypeptides as disclosed herein, but also variants thereof that have substantially the same activity as the wild-type genes and polypeptides.

The virulence gene products produced by the methods described above are used in high throughput assays to screen for inhibitory agents. The sources for potential agents to be screened are chemical compound libraries, fermentation media of *Streptomycetes*, other bacteria and fungi, and cell extracts of plants and other vegetations. For proteins with known enzymatic activity, assays are established based on the activity, and a large number of potential agents are screened for ability to inhibit the activity. For proteins that interact with another protein or nucleic acid, binding assays are established to measure such interaction directly, and the potential agents are screened for ability to inhibit the binding interaction.

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The use of different assays known in the art is contemplated according to this aspect of the invention. When the function of the virulence gene product is known or predicted by sequence similarity to a known gene product, potential inhibitors can be screened in enzymatic or other types of biological and/or biochemical assays keyed to the function and/or properties of the gene product. When the virulence gene product is known or predicted by sequence similarity to a known gene product to interact with another protein or nucleic acid, inhibitors of the interaction can be screened directly in binding assays. The invention contemplates a multitude of assays to screen and identify inhibitors of binding by the virulence gene product. In one example, the virulence gene product is immobilized and interaction with a binding partner is assessed in the presence and absence of a putative inhibitor compound. In another example, interaction between the virulence gene product and its binding partner is assessed in a solution assay, both in the presence and absence of a putative inhibitor compound. In both assays, an inhibitor is identified as a compound that decreases binding between the virulence gene product and its binding partner. Other assays are also contemplated in those instances wherein the virulence gene product binding partner is a protein. For example, variations of the di-hybrid assay are contemplated wherein an inhibitor of protein/protein interactions is identified by detection of a positive signal in a transformed or transfected host cell as described in PCT publication number WO 95/20652, published August 3, 1995.

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Candidate inhibitors contemplated by the invention include compounds selected from libraries of potential inhibitors. There are a number of different libraries used for the identification of small molecule modulators, including: (1) chemical

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libraries, (2) natural product libraries, and (3) combinatorial libraries comprised of random peptides, oligonucleotides or organic molecules. Chemical libraries consist of structural analogs of known compounds or compounds that are identified as "hits" or "leads" via natural product screening. Natural product libraries are collections of microorganisms, animals, plants, or marine organisms which are used to create mixtures for screening by: (1) fermentation and extraction of broths from soil, plant or marine microorganisms or (2) extraction of plants or marine organisms. Natural product libraries include polyketides, non-ribosomal peptides, and variants (non-naturally occurring) thereof. For a review, see Science 282:63-68 (1998). Combinatorial libraries are composed of large numbers of peptides, oligonucleotides, or organic compounds as a mixture. They are relatively easy to prepare by traditional automated synthesis methods, PCR, cloning, or proprietary synthetic methods. Of particular interest are peptide and oligonucleotide combinatorial libraries. Still other libraries of interest include peptide, protein, peptidomimetic, multiparallel synthetic collection, recombinatorial, and polypeptide libraries. For a review of combinatorial chemistry and libraries created therefrom, see Myers, Curr. Opin. Biotechnol. 8:701-707 (1997). Identification of modulators through use of the various libraries described herein permits modification of the candidate "hit" (or "lead") to optimize the capacity of the "hit" to modulate activity.

Still other candidate inhibitors contemplated by the invention can be designed and include soluble forms of binding partners, as well as binding partners as chimeric, or fusion, proteins. Binding partners as used herein broadly encompasses antibodies, antibody fragments, and modified compounds comprising antibody domains that are immunospecific for the expression product of the identified virulence gene.

Other assays may be used when a binding partner (*i.e.*, ligand) for the virulence gene product is not known, including assays that identify binding partners of the target protein through measuring direct binding of test binding partner to the target protein, and assays that identify binding partners of target proteins through affinity ultrafiltration with ion spray mass spectroscopy/HPLC methods or other physical and analytical methods. Alternatively, such binding interactions are evaluated indirectly using the yeast two-hybrid system described in Fields and Song, *Nature*, 340:245-246

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(1989), and Fields and Sternglanz, Trends in Genetics, 10:286-292 (1994), both of which are incorporated herein by reference. The two-hybrid system is a genetic assay for detecting interactions between two proteins or polypeptides. It can be used to identify proteins that bind to a known protein of interest, or to delineate domains or residues critical for an interaction. Variations on this methodology have been developed to clone genes that encode DNA-binding proteins, to identify peptides that bind to a protein, and to screen for drugs. The two-hybrid system exploits the ability of a pair of interacting proteins to bring a transcription activation domain into close proximity with a DNAbinding domain that binds to an upstream activation sequence (UAS) of a reporter gene, and is generally performed in yeast. The assay requires the construction of two hybrid genes encoding (1) a DNA-binding domain that is fused to a first protein and (2) an activation domain fused to a second protein. The DNA-binding domain targets the first hybrid protein to the UAS of the reporter gene; however, because most proteins lack an activation domain, this DNA-binding hybrid protein does not activate transcription of the reporter gene. The second hybrid protein, which contains the activation domain, cannot by itself activate expression of the reporter gene because it does not bind the UAS. However, when both hybrid proteins are present, the noncovalent interaction of the first and second proteins tethers the activation domain to the UAS, activating transcription of the reporter gene. When the virulence gene product (the first protein, for example) is already known to interact with another protein or nucleic acid, this assay can be used to detect agents that interfere with the binding interaction. Expression of the reporter gene is monitored as different test agents are added to the system; the presence of an inhibitory agent results in lack of a reporter signal.

When the function of the virulence gene product is unknown and no ligands are known to bind the gene product, the yeast two-hybrid assay can also be used to identify proteins that bind to the gene product. In an assay to identify proteins that bind to the first protein (the target protein), a large number of hybrid genes each encoding different second proteins are produced and screened in the assay. Typically, the second protein is encoded by a pool of plasmids in which total cDNA or genomic DNA is ligated to the activation domain. This system is applicable to a wide variety of proteins, and it is not even necessary to know the identity or function of the second binding protein. The

system is highly sensitive and can detect interactions not revealed by other methods; even transient interactions may trigger transcription to produce a stable mRNA that can be repeatedly translated to yield the reporter protein.

Other assays may be used to search for agents that bind to the target protein. One such screening method to identify direct binding of test ligands to a target protein is described in U.S. Patent No. 5,585,277, incorporated herein by reference. This method relies on the principle that proteins generally exist as a mixture of folded and unfolded states, and continually alternate between the two states. When a test ligand binds to the folded form of a target protein (i.e., when the test ligand is a ligand of the target protein), the target protein molecule bound by the ligand remains in its folded state. Thus, the folded target protein is present to a greater extent in the presence of a test ligand which binds the target protein, than in the absence of a ligand. Binding of the ligand to the target protein can be determined by any method which distinguishes between the folded and unfolded states of the target protein. The function of the target protein need not be known in order for this assay to be performed. Virtually any agent can be assessed by this method as a test ligand, including, but not limited to, metals, polypeptides, proteins, lipids, polysaccharides, polynucleotides and small organic molecules.

Another method for identifying ligands for a target protein is described in Wieboldt *et al.*, *Anal. Chem.*, 69:1683-1691 (1997), incorporated herein by reference. This technique screens combinatorial libraries of 20-30 agents at a time in solution phase for binding to the target protein. Agents that bind to the target protein are separated from other library components by centrifugal ultrafiltration. The specifically selected molecules that are retained on the filter are subsequently liberated from the target protein and analyzed by HPLC and pneumatically assisted electrospray (ion spray) ionization mass spectroscopy. This procedure selects library components with the greatest affinity for the target protein, and is particularly useful for small molecule libraries.

The inhibitors/binders identified by the initial screens are evaluated for their effect on virulence in *in vivo* mouse models of *P. multocida* infections. Models of bacteremia, endocarditis, septic arthritis, soft tissue abscess, or pneumonia may be utilized. Models involving use of other animals are also comprehended by the invention.

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For example, rabbits can be challenged with a wild type *P. multocida* strain before or after administration of varying amounts of a putative inhibitor/binder compound. Control animals, administered only saline instead of putative inhibitor/binder compound provide a standard by which deterioration of the test animal can be determined. Other animal models include those described in the <u>Animal and Plant Health Inspection Sevice</u>, <u>USDA</u>, January 1, 1994 Edition, §§113.69-113.70; Panciera and Corstvet, *Am. J. Vet. Res.* 45:2532-2537; Ames, *et al.*, *Can. J. Comp. Med.* 49:395-400 (1984); and Mukkur, *Infection and Immunity* 18:583-585 (1977). Inhibitors/binders that interfere with bacterial virulence are can prevent the establishment of an infection or reverse the outcome of an infection once it is established.

Any adjuvant known in the art may be used in the vaccine composition, including oil-based adjuvants such as Freund's Complete Adjuvant and Freund's Incomplete Adjuvant, mycolate-based adjuvants (e.g., trehalose dimycolate), bacterial lipopolysaccharide (LPS), peptidoglycans (i.e., mureins, mucopeptides, or glycoproteins such as N-Opaca, muramyl dipeptide [MDP], or MDP analogs), proteoglycans (e.g., extracted from Klebsiella pneumoniae), streptococcal preparations (e.g., OK432), Biostim<sup>™</sup> (e.g., 01K2), the "Iscoms" of EP 109 942, EP 180 564 and EP 231 039, aluminum hydroxide, saponin, DEAE-dextran, neutral oils (such as miglyol), vegetable oils (such as arachis oil), liposomes, Pluronic® polyols, the Ribi adjuvant system (see, for example GB-A-2 189 141), or interleukins, particularly those that stimulate cell mediated immunity. An alternative adjuvant consisting of extracts of Amycolata, a bacterial genus in the order Actinomycetales, has been described in U.S. Patent No. 4,877,612. Additionally, proprietary adjuvant mixtures are commercially available. The adjuvant used will depend, in part, on the recipient organism. The amount of adjuvant to administer will depend on the type and size of animal. Optimal dosages may be readily determined by routine methods.

The vaccine compositions optionally may include vaccine-compatible pharmaceutically acceptable (*i.e.*, sterile and non-toxic) liquid, semisolid, or solid diluents that serve as pharmaceutical vehicles, excipients, or media. Any diluent known in the art may be used. Exemplary diluents include, but are not limited to, polyoxyethylene sorbitan monolaurate, magnesium stearate, methyl- and

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propylhydroxybenzoate, talc, alginates, starches, lactose, sucrose, dextrose, sorbitol, mannitol, gum acacia, calcium phosphate, mineral oil, cocoa butter, and oil of theobroma.

The vaccine compositions can be packaged in forms convenient for delivery. The compositions can be enclosed within a capsule, caplet, sachet, cachet, gelatin, paper, or other container. These delivery forms are preferred when compatible with entry of the immunogenic composition into the recipient organism and, particularly, when the immunogenic composition is being delivered in unit dose form. The dosage units can be packaged, *e.g.*, in tablets, capsules, suppositories or cachets.

The vaccine compositions may be introduced into the subject to be immunized by any conventional method including, *e.g.*, by intravenous, intradermal, intramuscular, intramammary, intraperitoneal, or subcutaneous injection; by oral, sublingual, nasal, anal, or vaginal, delivery. The treatment may consist of a single dose or a plurality of doses over a period of time.

The invention also comprehends use of an attenuated bacterial strain of the invention for manufacture of a vaccine medicament to prevent or alleviate bacterial infection and/or symptoms associated therewith. The invention also provides use of inhibitors of the invention for manufacture of a medicament to prevent or alleviate bacterial infection and/or symptoms associated therewith.

The present invention is illustrated by the following examples. Example 1 describes constructions of *P. multocida* mutants. Example 2 relates to screening for *P. multocida* mutants. Example 3 addresses methods to determine virulence of the *P. multocida* mutants. Example 4 describes cloning of *P. multocida* virulence genes. Example 5 addresses identification of genes in other species related to *P. multocida* virulence genes. Example 6 describes construction of *A. pleuropneumoniae* mutants. Example 7 addresses screening for attenuated *A. pleuropneumoniae* mutants. Example 8 relates to identification of *A. pleuropneumoniae* virulence genes. Example 9 describes competition challenge of *A. pleuropneumoniae* mutants and wild type bacteria. Example 10 characterizes *A. pleuropneumoniae* genes identified. Example 11 addresses efficacy of *A. pleuropneumoniae* mutant to protect against wild type bacterial challenge.

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# Example 1 Construction of a Library of Tagged-Transposon *P. multocida* Mutants

A library of tagged-transposon mutants was constructed in parental vector pLOF/Km [Herrero, et al., J Bacteriol. 172:6557-67 (1990)] which has previously been demonstrated to be functional and random in P. multocida [Lee, et al., Vet Microbiol. 50:143-8 (1996)]. Plasmid pLOF/Km was constructed as a modification of suicide vector pGP704 and included a transposase gene under control of the Tac promoter as well as the mini-Tn10 transposable element encoding kanamycin resistance. Plasmid pTEF-1 was constructed as described below by modifying pLOF/Km to accept sequence tags which contained a semi-random [NK]<sub>35</sub> sequence.

Plasmid pLOF/Km was first modified to eliminate the unique KpnI restriction site in the multiple cloning region and then to introduce a new KpnI site in the mini-Tn10 region. The plasmid was digested with KpnI and the resulting overhanging ends were filled in with Klenow polymerase according to manufacturer's suggested protocol. Restriction digests and ligations described herein were performed according to manufacturer's suggested protocols (Gibco BRL, Gaithersburg, MD and Boehringer Mannheim, Indianapolis, IN). The blunt end product was self-ligated to produce a plasmid designated pLOF/Km--KpnI which was transformed into E.coli DH5α:λpir for amplification. E.coli DH5α: (λpir Φ80dlacZΔM15, recA1, endA1, gyrA96, thi-1,  $hsdR17(r_k^-, m_k^-, supE44, relA1, deoR, \Delta(lacZYA-argF)U169, was propagated at 37°C$ in Luria-Bertani (LB) medium. Plasmids were prepared using QIAGEN SpinPreps from QIAGEN Inc. (Santa Clarita, CA) and digested with SfiI which cuts at a unique site within the mini-Tn10 transposable element. A SfiI-KpnI-SfiI adaptor was prepared by annealing oligonucleotides TEF1 (SEQ ID NO: 86) and TEF3 (SEQ ID NO: 87) and the resulting double-stranded adapter was ligated into the SfiI site to create plasmid pTEF-1. Oligonucleotides TEF1 and TEF3 (as well as all other oligonucleotides described herein) were synthesized by Genosys Biotechnologies (The Woodlands, TX).

TEF1 5'-AGGCCGGTACCGGCCGCCT SEQ ID NO: 86

TEF3 5'-CGGCCGGTACCGGCCTAGG SEQ ID NO: 87

Unique sequence tags for insertion into the *Kpn*I site of pTEF-1 were prepared as follows. PCR was carried out to generate double stranded DNA tags using a GeneAmp XL PCR Kit (PE Applied Biosystems, Foster City, CA) under conditions including 250 µM each dNTP, 1.5 mM Mg(OAc)<sub>2</sub>, 100 pmol each primer TEF14 (SEQ ID NO: 88) and TEF15 (SEQ ID NO: 89), 1 ng TEF26 (SEQ ID NO: 90) as template DNA and 2.5 units recombinant *Tth* DNA Polymerase XL.

TEF14	5'-CATGGTACCCATTCTAAC	SEQ ID NO: 88							
TEF15	5'-CTAGGTACCTACAACCTC	SEQ ID NO: 89							
TEF26		SEQ ID NO: 90							
5'-CTAGG	TACCTACAACCTCAAGCTT-[NK] <sub>35</sub>	5-							
AAGCTTGGTTAGAATGGGTACCATG									

Reaction conditions included an initial incubation at 95°C for one minute, followed by thirty cycles of 30 seconds at 95°C, 45 seconds at 45°C, and 15 seconds at 72°C, followed by a final incubation at 72°C for two minutes. The PCR products were digested with KpnI and purified using a QIAGEN Nucleotide Removal Kit (QIAGEN, Inc., Chatsworth, GA) according to the manufacturer's suggested protocol. The unique tag sequences were ligated into the mini-Tn10 element of linearized pTEF-1, previously digested with KpnI and dephosphorylated with calf intestinal alkaline phosphatase (Boehringer Mannheim) using standard procedures. The resulting plasmid library was transformed into E.coli DH5 $\alpha$ : $\lambda$ pir. Colony blot analysis was performed according to the DIG User's Guide (Boehringer-Mannheim) with hybridization and detection performed as follows.

Hybridizations were essentially performed according to the Genius Non-Radioactive User's Guide (Boehringer Mannheim Biochemicals), the product sheet for the DIG-PCR labeling kit (Boehringer Mannheim Biochemicals), and the product sheet for CSPD (Boehringer Mannheim Biochemicals). For preparation of probes, a 100  $\mu$ l primary PCR reaction was set up using Amplitaq PCR buffer (PE Applied Biosystems),

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 $200~\mu\text{M}$  dNTPs, 140~pmol each of primers TEF5 (SEQ ID NO: 91) and TEF6 (SEQ ID NO: 92),  $2~\text{mM}~\text{MgCl}_2$ , 2.5~units~Amplitaq (PE Applied Biosystems) and 1~ng~of~plasmid DNA.

5	TEF5	5'-TACCTACAACCTCAAGCT	SEQ ID NO: 91
)		J-TACCTACIA CCT CARTGET	DEQ ED TIONS

TEF6 5'-TACCCATTCTAACCAAGC SEQ ID NO: 92

Cycle conditions included an initial incubation at 95°C for two minutes, followed by 35 cycles of 95°C for 30 seconds, 50°C for 45 seconds, 72°C for 15 seconds and a final incubation at 72°C for three minutes. The amplification products were separated using electrophoresis on a 2% - 3:1 NuSieve GTG (FMC BioProducts, Rockland, ME, USA):Agarose gel and the 109 bp product was excised and purified. Gel extractions were carried out using a QIAGEN Gel Extraction kit (QIAGEN). Approximately 15 ng of the primary product was labeled in a 50 µl PCR reaction using the DIG PCR Kit, 50 pmol each of primers TEF24 and TEF25, and a 1:1 mix of DIG Probe Synthesis Mix with 2 mM dNTP stock solution.

TEF24 5'-TACCTACAACCTCAAGCTT SEQ ID NO: 93

TEF25 5'-TACCCATTCTAACCAAGCTT SEQ ID NO: 94

PCR conditions included an initial incubation at 95°C for four minutes, followed by 25 cycles of 95°C for 30 seconds, 50°C for 45 seconds, 72°C for 15 seconds and a final incubation at 72°C for three minutes. The labeled PCR product was digested with *Hin*dIII in a total reaction volume of 90 µl and purified from the constant primer arms using a 2% - 3:1 NuSieve GTG (FMC BioProducts):Agarose gel. The region containing the labeled variable tag was excised and the entire gel slice was dissolved and denatured in 10 ml of DIG EasyHyb at 95°C for ten minutes.

Dot blots were prepared using a Hybond<sup>®</sup>-N<sup>+</sup> membrane (Amersham-Pharmacia Biotech). Target DNA for each tag was prepared in 96 well plates using

approximately 30 ng of PCR product. An equal volume of 0.1 N NaOH was added to denature the sample and each sample was applied to the membrane with minimal vacuum using a Minifold I<sup>TM</sup> Dot-Blot Apparatus from Schleicher and Schuell (Keene, NH, USA). Each well was washed with 150 µl of Neutralization Solution (0.5 M Tris /3 M NaCl, pH 7.5) and 150 µl of 2X SSC. Membranes were UV-crosslinked in a Stratalinker (Stratagene, La Jolla, CA, USA) and prehybridized for one hour in 20 mls DIG EasyHyb Buffer at 42°C. The denatured probe was added and hybridization carried out overnight at 42°C. The membrane was washed two times in 2X SSC containing 0.1% SDS for five minutes each wash. Two high stringency washes were performed in 50 ml of pre-warmed 0.1X SSC buffer containing 0.1% SDS at 68°C for 15 minutes before proceeding with standard Genius Detection protocols (Genius Manual ).

It is desirable to use a non-radioactive detection system for safety, lower cost, ease of use, and reduction of hazardous materials. In initial experiments using similar procedures previously described [Mei, et al., Mol Microbiol. 26:399-407 (1997)], unacceptable background levels of hybridization were obtained in negative controls. In order to decrease background, tag length was increased by 30 bp to a total of 70, amplification primers were lengthened to include all sequence flanking the variable region, a lower concentration of dig-dUTP was used, and the conserved sequences flanking the sequence tag region were removed by gel purification. Most significantly, PCR was used to generate [NK]<sub>35</sub> sequence tags as the target DNA in dot blots rather than the entire plasmids containing the tagged transposons after detecting background hybridization from the transposon itself. Using these modifications background was eliminated making chemiluminescent/non-radioactive screening more effective.

Approximately four hundred different transformants resulting from the ligation of pTEF-1 with the PCR generated sequence tags were screened by colony blot and the 96 strongest hybridizing colonies were assembled into microtiter plates for further use. Even though the likelihood of duplicated tags was very low, half of the plate of master tags was probed against the other to confirm that no tags were duplicated. The plasmids containing these tags were purified and transformed into *E.coli* S17-1:λpir (pir, recA, thi, pro, hsd, (r-m+), RP4-2, (Tc::Mu), (Km::Tn7), [TmpR], [SmR]), and the transformed bacteria propagated at 37°C in Luria-Bertani (LB) medium. Each of the 96

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E.coli S17-1:λpir transformants containing the tagged plasmid pTEF-1 was used in conjugative matings to generate transposon mutants of P. multocida. P. multocida strain TF5 is a spontaneous nalidixic acid resistant mutant derived from UC6731, a bovine clinical isolate. P. multocida strains were grown on brain heart infusion (BHI) media (Difco Laboratories, Detroit, MI, USA) at 37°C and in 5% CO<sub>2</sub> when grown on plates. Matings were set up by growing each E.coli S17-1:λpir /pTEF1:[NK]<sub>35</sub> clone and the TF5 strain to late log phase. Fifty  $\mu l$  of culture for each tagged-pTEF-1 clone was mixed with 200 µl of the TF5 culture and 50 µl of each mating mixture was spotted onto 0.22 TM filters previously placed on BHI plates containing 100 mM IPTG and 10 mM MgSO<sub>4</sub>. Following overnight incubation at 37°C with 5% CO<sub>2</sub>, mating mixtures were washed off of each filter into 3 ml of PBS and 25  $\mu$ l of each was plated onto BHIN  $^{50}$ K  $^{100}$ plates. Following selective overnight growth, colonies were assembled into microtiter plates by toothpick transfer into 200 µl BHIN50K50 making sure that each well in a microtiter plate always contained a transposon mutant with the same sequence tag. Following overnight growth, 50 µl of 75% glycerol was added to each well and plates were stored frozen at -80°C.

Nineteen pools were assembled by transferring the transposon mutants to microtiter plates making sure that each well contained a transposon mutant with the appropriate tag for that well. In other words, a specific well in each microtiter plate always contained a transposon mutant with the same sequence tag even though the location of the transposon within those mutants may be different.

### **Example 2 Murine Screening for Attenuated** *P. multocida* **Mutants**

Nineteen pools of *Pasteurella multocida* transposon mutants were screened using a murine model of septicemia. Frozen plates of pooled *P. multocida* transposon mutants were removed from -80°C storage and subcultured by transferring 10 µl from each well to a new 96 well round bottom plate (Corning Costar, Cambridge, MA, USA) containing 200 µl of brain heart infusion (DIFCO) with 50 µg/ml nalidixic acid (Sigma) and 50 µg/ml kanamycin (Sigma) (BHIN<sup>50</sup>K<sup>50</sup>). Plates were incubated without shaking overnight at 37°C in 5% CO<sub>2</sub>. Overnight plates were subcultured by transferring 10 µl from each well to a new flat bottomed 96-well plate (Corning Costar)

containing 100 µ1 of BHI per well and incubating at 37°C with shaking at approximately 150 rpm. The OD<sub>540</sub> was monitored using a micro-titer plate reader. At an OD<sub>540</sub> of approximately 0.2 to 0.25, each plate was pooled to form the "input pool" by combining 100 µ1 from each of the wells of the micro-titer plate. The culture was diluted appropriately in BHI to doses of approximately 10<sup>4</sup>, 10<sup>5</sup>, 10<sup>6</sup> CFU/ml and 0.2 ml of each dilution was used to infect female 14-16 g BALB/c mice by intraperitoneal administration. At two days post-infection, one or two surviving mice were euthanized and the spleens harvested. The entire spleen was homogenized in 1.0 ml sterile 0.9 % saline. Dilutions of the homogenate from 10<sup>-2</sup> to 10<sup>-5</sup> were prepared and plated onto BHIN<sup>50</sup>K<sup>50</sup> plates. Following overnight growth, at least 20,000 colonies were pooled in 10 mls BHI broth to form the "recovered pool" and 0.5 ml of the recovered pool was centrifuged at 3,500 X g and the pellet used to prepare genomic DNA according to a previously described protocol [Wilson, *In* F. M. Ausubel, *et al.*,(ed.), <u>Current Protocols in Molecular Biology</u>, vol. 1. John Wiley and Sons, New York, p. 2.4.1-2.4.5. (1997)].

Initial experiments with virulent wild-type *P. multocida* indicated that organisms could be recovered from the spleen, lungs, kidneys, and liver indicating a truly septicemic model of infection. Dot blots for both the "input" and "recovered" pools were performed as described in Example 1 and evaluated both by visual inspection and by semi-quantitative analysis. Hybridization was carried out as described in Example 1 except that 5 µg of genomic DNA from input and recovered pools was used as template. Semi-quantitative analysis indicates whether a significant reduction in a single clone has occurred. If a mutant is unable to survive within the host, then the recovered signal should be very low compared to the input signal yielding a high input/recovered ratio. Most mutants will grow as well *in vivo* as *in vitro* and therefore a ratio of their signals should be approximately equal to 1. Clones selected by quantitative analysis as being highly reduced in the recovered pool were selected for further study. Additional clones with questionable input/recovered ratios were also selected after visually evaluating films made from the dot blots.

## Example 3 Determination of Virulence for *P. multocida* Candidate Mutants

Each potential mutant which exhibited reduced recovery from splenic tissue was isolated from the original pool plate and used individually in a challenge experiment to verify and roughly estimate the attenuation caused by the transposon mutation. Individual candidate mutants from *in vivo* screens were grown on Sheep Blood Agar plates overnight in 5% CO<sub>2</sub> at 37°C. Approximately six colonies of each mutant were inoculated into BHI broth and allowed to grow for six hours. Dilutions were prepared and five mice each were infected as described above with 10², 10³, 10⁴ and 10⁵ CFU each. Attenuation was determined by comparing mortality after six days relative to the wild type. Surviving mice were presumed to be protected and then challenged with a dose of wild type *P. multocida* at a concentration approximately 200-fold greater than the LD<sub>50</sub> for the wild type strain. Survival rate was then determined for each challenged group of mice.

Results indicated that 62 of 120 potential transposon mutants were attenuated, having an approximate  $LD_{50}$  of at least 10 fold higher than the wild type strain. The clones and their approximate  $LD_{50}$  values are listed in Table 1. A control experiment with the wild type strain was run in parallel with each set of challenges and in all cases mortality in wild type-challenged groups was 100%.

In addition to  $LD_{50}$  values, Table 1 also provides data from vaccination and challenge experiments. Briefly, groups of mice (n = 5 to 10) were vaccinated by intraperitoneal injection with the individual P. multocida strains shown in Table 1 at a dose that was approximately 200 times greater than the  $LD_{50}$  of the virulent, wild type strain. Animals were observed for 28 days after which mortality figures were calculated.

Table 1

P. multocida Virulence Genes

Nucleotide SEQ ID NO:	Representative Isolate	PossibleGene Function	Vaccination # survivors/total	Challenge # survivors/total	LD <sub>50</sub>	
	wild type	-	0/10	-	<10	
23	PM1B1	guaB	10/10, 10/10, 10/10	9/10, 9/10	4.3 x 106	
11	PM1D1	dsbB	10/10, 5/10	10/10, 5/5	8.4 x 104	
3	PM1BD7	atpG	5/5, 10/10	10/10	>3 x 105	
74	PM1BE11	vhcJ (HI0145)	10/10	5/10	>2 x 105	
70	PM1BF6	yabK (HI1020)	3/5, 8/10	9/9	>2 x 105	
19	PM2G8	fhaC	4/5, 9/10	9/9	>4 x 105	

	Nucleotide	Representative	PossibleGene	Vaccination	Challenge	$LD_{50}$
	SEQ ID NO:	Isolate	Function	# survivors/total	# survivors/total	
	76	PM3C9	yiaO (HI0146)	3/5		>6 x 105
	118	PM3G11	UnkO	4/5, 10/10	10/10	>3 x 105
	31	PM7B4	iroA (UnkB)	0/5		
	17	PM4C6	fhaB (fhaB2)	2/5, 10/10, 9/10	10/10, 9/9	>3 x 106
5	9	PM4G10-T9	dnaA	4/5		>5 x 105
5	1	PM4D5-T5	atpB	5/5		>4 x 105
	53	PM4D5-T1	UnkC2	5/5		>4 x 105
	15	PM4F2	fhaB (fhaB1)	3/5, 6/10, 10/10	6/6, 10/10	>3 x 105
	41	PM5F7	mreB	4/5		1 x 103
10	7	PM5E2	devB	0/5, 3/10	2/3	?
	68	PM6H5-T1	xylA	5/5		>3 x 105
	78	PM6H8	yigF (HI0719)	5/5, 9/10	9/9	>3 x 105
	108	PM7D12	pnp	5/5, 9/10	9/9	
	51	PM8C1R1-T2	UnkC1	5/5		~6 x 105
15	37	PM8C1-T3	mglB	5/5		~6 x 105
10	58	PM8C1R1-T6	UnkD1	5/5		~6 x 10
	45	PM10H7	purF (HI1207)	3/5, 8/10, 8/10	8/8, 8/8	>3 x 10
	25	PM10H10-T2	HI1501	5/5		>1 x 10 <sup>4</sup>
	72	PM11G8-T2	ygiK	5/5		>2.4 x 10
20	21	PM11G8-T4	greA	5/5		>2.4 x 10
<b>-</b> 0	84	PM12H6	yyam	3/5, 0/10		~2.2 x 10
			(HI0687)			
	33	PM15G8-T2	kdtB	5/5		>1.2 x 10
	116	PM15G8-T1	UnkK	5/5		>1.2 x 10
	104	PM16G11-T1	hmbR	3/5		>1.9 x 10
25	29	PM16G11-T2	hxuC	3/5		>1.9 x 10
	35	PM16H8	lgtC	5/5, 10/10	10/10	>2.4 x 10
	80	PM16H3	yleA (HI0019)	5/5, 10/10		> 2.0 x 1
	49	PM17H6-T1	sopE	4/5		~6 x 10
	120	PM17H6	UnkP	4/5		~6 x 10
30	5	PM18F5-T8	cap5E	5/5		>2.4 x 10
	82	PM18F5-T10	yojB (HI0345)	5/5		>2.4 x 10
	13	PM19A1	exbB	5/5, 10/10	10/10	>1.2 x 10
	112	PM19D4	rci	5/5, 8/10	8/8	~1.6 x 10
	39	PM20A12	mioC	3/5, 8/10	8/8	~2 x 10
			(HI0669)	7/7 10/10	10/10	>00:41
35	60	PM20C2	UnkD2	5/5, 10/10	10/10	>8.2 x 10

Vaccination

LD.

Challenge

**Example 4**Cloning and Identification of Genes Required for *P. multocida* Virulence

Each transposon mutant which was verified to be attenuated was analyzed further to determine the identity of the disrupted open reading frame. DNA from each mutant was amplified, purified, and digested with restriction enzymes that were known not to cut within the transposon and generally produced 4-8 kb fragments that hybridized with the transposon. Using selection for kanamycin resistance encoded by the transposon, at least one fragment for each transposon mutant was cloned.

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Southern hybridization with multiple restriction enzymes was performed for each attenuated mutant using a labeled 1.8 kb *MluI* fragment from pLOF/Km as a probe to identify a suitably sized fragment for cloning. The mini-Tn10 element and

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flanking DNA from each mutant was cloned into pUC19 and the flanking sequence determined using internal primers TEF32 and TEF40, primer walking and in some cases universal pUC-19 primers.

TEF-32 GGCAGAGCATTACGCTGAC SEQ ID NO: 95
TEF-40 GTACCGGCCAGGCGGCCACGCGTATTC SEQ ID NO:96

Sequencing reactions were performed using the BigDye<sup>TM</sup> Dye Terminator Chemistry kit from PE Applied Biosystems (Foster City, CA) and run on an ABI Prism 377 DNA Sequencer. Double stranded sequence for putative interrupted open reading frames was obtained for each clone. Sequencer 3.0 software (Genecodes, Corp., Ann Arbor, MI) was used to assemble and analyze sequence data. GCG programs [Devereux, *et al.*, 1997. Wisconsin Package Version 9.0, 9.0 ed. Genetics Computer Group, Inc., Madison] were used to search for homologous sequences in currently available databases.

In 37% of the clones that were identified as being attenuated, there were multiple insertions of the mini-Tn10 transposable element. Each insertion including its flanking sequence was cloned individually into pGP704 and mated into the wild-type strain to produce new mutants of *P. multocida*, each carrying only one of the multiple original insertions. Individual mutants were retested individually to determine the insertion responsible for the attenuated phenotype. The nucleotide sequence of the disrupted, predicted open reading frame was determined by sequencing both strands, and the predicted amino acid sequence was used to search currently available databases for similar sequences. Sequences either matched known genes, unknown genes, and hypothetical open reading frames previously sequenced or did not match any previously identified sequence. For those genes having homology to previously identified sequences, potential functions were assigned as set out in Table 1.

### **Example 5 Identification of Related Genes in Other Species**

In separate experiments, STM was also performed using *Actinobacillus* pleuropneumoniae (App). One of the App strains contained an insertion in a gene that was sequenced (SEQ ID NO: 97) and identified as a species homolog of the *P. multocida* 

atpG gene. This result suggested the presence in other bacterial species of homologs to previously unknown P. multocida genes that can also be mutated to produce attenuated strains of the other bacterial species for use in vaccine compositions. In order to determine if homologs of other P. multocida genes exists in other bacterial species, Southern hybridization was performed on genomic DNA from other species using the A. pleuropneumoniae atpG gene as a probe.

Actinobacillus pleuropneumoniae, Pasteurella haemolytica (Ph), P. multocida, and Haemophilus somnus (Hs) genomic DNA was isolated using the CTAB method and digested with EcoRI and HindIII for two hours at 37°C. Digested DNA was separated on a 0.7% agarose gel at 40V in TAE buffer overnight. The gel was immersed sequentially in 0.1 M HCL for 30 minutes, twice in 0.5 M NaOH/1.5 M NaCl for 15 minutes each, and twice in 2.5 M NaCl/1 M Tris, pH 7.5. The DNA was transferred to nitrocellulose membranes (Amersham Hybond N<sup>+</sup>) overnight using 20X SSC buffer (3 M NaCl/0.3 M sodium citrate). The DNA was crosslinked to the membrane using a UV Stratalinker on autocrosslink setting (120 millijoules). The membrane was prehybridized in 5X SSC/1% blocking solution/0.1% sodium lauroyl sarcosine/0.02% SDS at 50°C for approximately seven hours and hybridized overnight at 50°C in the same solution containing a PCR generated atgG probe.

The probe was prepared using primers DEL-1389 (SEQ ID NO: 98) and TEF-46 (SEQ ID NO: 99) in a with a GeneAmp XL PCR kit in a GeneAmp PCR System 2400. Template was genomic *A. pleuropneumoniae* DNA.

DEL-1389 TCTCCATTCCCTTGCTGCGGCAGGG SEQ ID NO: 98
TEF-46 GGAATTACAGCCGGATCCGGG SEQ ID NO: 99

The PCR was performed with an initial heating step at 94°C for five minutes, 30 cycles of denaturation t 94°C for 30 sec, annealing at 50°C for 30 sec, and elongation at 72°C for three minutes, and a final extension step at 72°C for five minutes. The amplification products were separated on an agarose gel, purified using a QIAquick gel purification kit (QIAGEN), and labeled using a DIG-High Primer kit (Boehringer Mannheim). The blot was removed from the hybridization solution and rinsed in 2X SSC and washed two times for five minutes each wash in the same buffer. The blot was then washed two

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times for 15 minutes each in 0.5X SSC at 60°C. Homologous bands were visualized using a DIG Nucleic Acid Detection Kit (Boehringer Mannheim).

Single bands were detected in *Pasteurella haemolytica, Haemophilus* somnus and A. pleuropneumoniae using EcoRI digested DNA. Two bands were detected using EcoRi digested DNA from *Pasteurella multocida*.

### Example 6 Construction of a Library of Tagged-Transposon *P. multocida* Mutants

Transposon mutagenesis using pLOF/Km has previously been reported to be functional and random in *A. pleuropneumoniae* [Tascon, *et al., J Bacteriol. 175*:5717-22 (1993)]. To construct tagged transposon mutants of *A. pleuropneumoniae*, each of 96 *E. coli* S17-1:λpir transformants containing pre-selected tagged plasmids (pTEF-1:[NK]<sub>35</sub>) was used in conjugative matings to generate transposon mutants of *A. pleuropneumoniae* strain AP225, a serotype 1 spontaneous nalidixic acid resistant mutant derived from an in vivo passaged ATCC 27088 strain. *A. pleuropneumoniae* strains were grown on Brain Heart Infusion (BHI) (Difco Laboratories, Detroit, MI) media with 10 μg/ml B-nicotinamide adenine dinucleotide (V<sup>10</sup>), (Sigma, St. Louis, Missouri) at 37°C and in 5% CO<sub>2</sub> when grown on plates. *E.coli* S17-1:λpir (λpir, *recA*, *thi*, *pro*, *hsdR*(r<sub>k</sub>, m<sub>k</sub>+), RP4-2, (Tc<sup>R</sup>::Mu), (Km<sup>R</sup>::Tn7), [Tmp<sup>R</sup>], [Sm<sup>R</sup>]) was propagated at 37°C in Luria-Bertani (LB) medium. Antibiotics when necessary were used at 100 μg/ml ampicillin (Sigma), 50 μg/ml nalidixic acid (N<sup>50</sup>)(Sigma), and 50 (K<sup>50</sup>) or 100 (K<sup>100</sup>) μg/ml of kanamycin (Sigma).

Matings were set up by growing each *E. coli* S17-1:λpir/pTEF1:[NK]<sub>35</sub> clone and the AP225 strain to late log phase. A 50 μl aliquot of culture for each tagged-pTEF-1 clone was mixed with 150 μl of the APP225 culture, and then 50 μl of each mating mixture was spotted onto 0.22 μM filters previously placed onto BHIV<sup>10</sup> plates containing 100 μM IPTG and 10 mM MgSO<sub>4</sub>. Following overnight incubation at 37°C with 5% CO<sub>2</sub>, mating mixtures were washed off of each filter into 2 ml of PBS and 200 μl of each was plated onto BHIV<sup>10</sup>N<sup>50</sup>K<sup>100</sup> plates. After selective overnight growth, colonies were assembled into microtiter plates by toothpick transfer into 200 μl BHIV<sup>10</sup>N<sup>50</sup>K<sup>50</sup> making sure that each well in a microtiter plate always contained a

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transposon mutant with the same sequence tag. Following overnight growth, 50 µl of 75% glycerol was added to each well and plates were stored frozen at -80°C.

APP does not appear to have as much bias towards multiple insertions of the mini-Tn10 element as did P. multocida. Only approximately 3% of the mutants were determined to contain multiple insertions, which is in agreement with the 4% previously reported [Tascon, et al., JBacteriol. 175:5717-22 (1993)]. A problem in APP consisted of identifying numerous mutants (discussed below) containing insertions into 23S RNA regions: 28 total mutants with insertions into 13 unique sites. This may indicate that 23S RNA contains preferential insertion sites and that the growth of APP is affected by these insertions enough to result in differential survival within the host. Southern blot analysis using an APP 23S RNA probe suggests that APP may contain only three ribosomal operons as compared to five in H. influenzae [Fleischmann, et al., Science 269:496-512 (1995)] and seven complete operons in E. coli [Blattner, et al., Science 277:1453-1474 (1997)]. This site preference and its effect on growth rate may be a significant barrier to "saturation mutagenesis" since a significant number of clones will contain insertions into these rRNAs and large volume screening will be necessary to obtain additional unique attenuating mutations.

### **Example 7 Porcine Screening for Attenuated** *A. pleuropneumoniae* **Mutants**

Twenty pools of *A. pleuropneumoniae* transposon mutants, containing a total of approximately 800 mutants, were screened using a porcine intratracheal infection model. Each pool was screened in two separate animals.

Frozen plates of pooled *A. pleuropneumoniae* transposon mutants were removed from -80°C storage and subcultured by transferring 20 μl from each well to a new 96 well round bottom plate (Corning Costar, Cambridge, MA, USA) containing 180 μl of BHIV<sup>10</sup>N<sup>50</sup>K<sup>50</sup>. Plates were incubated without shaking overnight at 37°C in 5% CO<sub>2</sub>. Overnight plates were then subcultured by transferring 10 μl from each well to a new flat bottomed 96 well plate (Corning Costar) containing 100 μl of BHIV<sup>10</sup> per well and incubating at 37°C with shaking at 150 rpm. The OD<sub>562</sub> was monitored using a microtiter plate reader. At an OD<sub>562</sub> of approximately 0.2 to 0.25, each plate was pooled to form the "input pool" by combining 100 μl from each of the wells of the microtiter

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TEF66

plate. The culture was diluted appropriately in BHI to approximately 2 X 10<sup>6</sup> CFU/ml. For each diluted pool, 4.0 ml was used to infect 10-20 kg SPF pigs (Whiteshire-Hamroc, Albion, IN) by intratracheal administration using a tracheal tube. At approximately 20 hours post-infection, all surviving animals were euthanized and the lungs removed. Lavage was performed to recover surviving bacteria by infusing 150 mls of sterile PBS into the lungs, which were then massaged to distribute the fluid. The lavage fluid was recovered, and the process was repeated a second time. The lavage fluid was centrifuged at 450 x g for 10 minutes to separate out large debris. Supernatants were then centrifuged at 2,800 x g to pellet the bacteria. Pellets were resuspended in 5 mls BHI and plated in dilutions ranging from 10<sup>-2</sup> to 10<sup>-5</sup> onto BHIV<sup>10</sup>N<sup>50</sup>K<sup>50</sup> plates. Following overnight growth, at least 100,000 colonies were pooled in 10 mls BHI broth to form the "recovered pools". A 0.7 ml portion of each recovered pool was used to prepare genomic DNA by the CTAB method [Wilson, *In* Ausubel, *et al.*, (eds.), Current Protocols in Molecular Biology, vol. 1. John Wiley and Sons, New York, p. 2.4.1-2.4.5 (1997)].

Recovery from the animals routinely was in the  $10^8\,\mathrm{CFU}$  range from lung lavage.

Dot blots were performed and evaluated both by visual inspection and by semi-quantitative analysis as described previously. All hybridizations and detections were performed as described. Briefly, probes were prepared by a primary PCR amplification, followed by agarose gel purification of the desired product and secondary PCR amplification incorporating dig-dUTP. Oligonucleotides including TEF5, TEF6, TEF24, TEF25, TEF48 and TEF62, were synthesized by Genosys Biotechnologies (The Woodlands, TX). Primers TEF69, TEF65, and TEF66 were also used for inverse PCR reactions and sequencing.

TEF69	GACGTTTCCCGTTGAATATGGCTC	SEQ ID NO: 166
TEF65	GCCGGATCCGGGATCATATGACAAGA	SEQ ID NO: 167

GACAAGATGTGTATCCACCTTAAC

The labeled PCR product was then digested with *Hin*dIII to separate the constant primer arms from the unique tag region. The region containing the labeled variable tag was excised and the entire gel slice was then dissolved and denatured in DIG

**SEQ ID NO: 168** 

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EasyHyb. Dot blots were prepared and detected using the standard CSPD detection protocol. Film exposures were made for visual evaluation, and luminescent counts per second (LCPS) were determined for each dot blot sample. The LCPS $_{input}$ / LCPS $_{recovered}$  ratio for each mutant was used to determine mutants likely to be attenuated.

Clones selected as being present in the input pool but highly reduced in the recovered pool were selected for further study. Additional clones with questionable input/recovered ratios were also selected after visually evaluating films made from the dot blots. A total of 110 clones were selected.

## **Example 8 Identification of** *A. pleuropneumoniae* **Virulence Genes**

A partial flanking sequence was determined for each of the 110 mutants by inverse PCR and direct product sequencing. Inverse PCR was used to generate flanking DNA products for direct sequencing as described above. Sequencing reactions were performed using the BigDye<sup>tm</sup> Dye Terminator Chemistry kit from PE Applied Biosystems (Foster City, CA) and run on an ABI Prism 377 DNA Sequencer. Sequencher 3.0 software (Genecodes, Corp., Ann Arbor, MI) was used to assemble and analyze sequence data. GCG programs [Devereux and Haeberli, Wisconsin Package Version 9.0, 9.0 ed. Genetics Computer Group, Inc., Madison (1997)] were used to search for homologous sequences in currently available databases.

Table 2 shows the *A. pleuropneumoniae* genes identified and extent to which open reading frames were determinable. Sequence identification numbers are provided for nucleotide sequences as well as deduced amino acid sequences where located.

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Table 2

A. pleuropneumoniae Open Reading Frames

	Complete Open Reading	ng Frame	NO Start Codon - Stop Codon				
5	atpH	SEQ ID NO: 134	dksA	SEQ ID NO: 136			
	aptG	SEQ ID NO: 132	dnaK	SEQ ID NO: 138			
	exbB	SEQ ID NO: 140	HI0379	SEQ ID NO: 144			
	OmpP5	SEQ ID NO: 152					
	OmpP5-2	SEQ ID NO: 150	NO Start Codon - NO	Stop Codon			
10	tig	SEQ ID NO: 160	pnp	SEQ ID NO: 154			
	fkpA	SEQ ID NO: 142	apvA-or 1	SEQ ID NO: 122			
	hupA	SEQ ID NO: 146	apvA-or 2	SEQ ID NO: 124			
	rpmF	SEQ ID NO: 158	apvB	SEQ ID NO: 126			
			apvD	SEQ ID NO: 130			
15	Start Codon - NO Stop	<u>Codon</u>					
	lpdA	SEQ ID NO: 148	RNA or Noncoding Se	equences			
	potD	SEQ ID NO: 156	tRNA-leu	SEQ ID NO: 162			
	yaeE	SEQ ID NO: 164	tRNA-glu	SEQ ID NO: 163			
	apvC	SEQ ID NO: 128					

The putative identities listed in Table 3 (below, Example 9) were assigned by comparison with bacterial databases. The 110 mutants represented 35 groups of unique transposon insertions. The number of different mutations per loci varied, with some clones always containing an insertion at a single site within an ORF to clones containing insertions within different sites of the same ORF. Three multiple insertions were detected in the 110 mutants screened as determined by production of multiple PCR bands and generation of multiple sequence electropherograms.

#### Example 9

### Competition Challenge of A. pleuropneumoniae Mutants with Wild Type APP225

A representative clone from each of the unique attenuated mutant groups identified above that was absent or highly reduced in the recovered population was isolated from the original pool plate and used in a competition challenge experiment with the wild type strain (AP225) to verify the relative attenuation caused by the transposon mutation. Mutant and wild type strains were grown in BHIV<sup>10</sup> to an  $OD_{590}$  of 0.6 - 0.9. Approximately  $5.0 \times 10^6$  CFU each of the wild type and mutant strains were added to 4 mls BHI. The total 4 ml dose was used infect a 10-20 kg SPF pig by intratracheal

administration with a tracheal tube. At approximately 20 hours post-infection, all surviving animals were euthanized and the lungs removed. Lung lavages were performed as described above. Plate counts were carried out on BHIV<sup>10</sup>N<sup>50</sup> and BHIV<sup>10</sup>N<sup>50</sup>K<sup>100</sup> to determine the relative numbers of wild type to mutant in both the input cultures and in the lung lavage samples. A Competitive Index (CI) was calculated as the [mutant CFU / wild type CFU]<sub>recovered</sub>.

Of the 35 potential transposon mutants, 22 were significantly attenuated, having a competitive index (CI) of less than 0.2. A transposon mutant that did not seem to be attenuated based on the STM screening results was chosen from one of the pools as a positive control. This mutant had a CI in vivo of approximately 0.6. An in vitro competition was also done for this mutant resulting in a CI of 0.8. The mutant was subsequently determined to contain an insertion between 2 phenylalanine tRNA's.

Competitive indices for unique attenuated single-insertion mutants are listed in Table 3. Competitive indices for *atpG*, *pnp*, and *exbB* App mutants indicated that the mutants were unable to compete effectively with the wild type strains and were therefore attenuated.

Table 3 Virulence and Proposed Function of *A. pleuropneumoniae* Mutants

Mutant	Similarity	Putative or Known Functions	C.I.
AP20A6	atpH	ATP synthase	.009
AP7F10	atpG	ATP synthase	.013
AP17C6	lpdA	dihydrolipoamide dehydrogenase	.039
AP11E7	exbB	transport of iron compounds	.003,.003,.006
AP3H7	potD	Spermidine/putrescine transport	.308
AP8H6	OmpP5	Adhesin / OmpA homolog	.184
AP18H8	OmpP5-2	Adhesin / OmpA homolog	.552
AP13E9	tig	Peptidyl-prolyl isomerase	.050
AP13C2	fkpA	Peptidyl-prolyl isomerase	<.001
AP15C11	pnp	Polynucleotide phosphorylase	.032
AP18F12	hupA	Histone – like protein	.001
AP20F8	dksA	Dosage dependent suppressor of dnaK mutations	.075
AP5G4	dnaK	Heat shock protein – molecular chaperone	.376

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AP17C9	tRNA-leu	Protein Synthesis (gene regulation?)	.059
AP5D6	tRNA-glu	Protein Synthesis	.055
AP18B2	rpmF	Protein Synthesis	.112
AP10E7	yaeA	Unknown	.001
AP19A5	HI0379	Unknown	.061
AP10C10	apvA	Unknown	.157
AP18F5	арvВ	Unknown	.103
AP2A6	apvC	Unknown	.091
AP2C11	apvD	Unknown	.014

Accuracy of the CI appeared to be very good as the *exbB* mutant was competed within three different animals yielding CI's of 0.003, 0.003 and 0.006. The use of a Competitive Index number to assign attenuation based upon one competition in a large animal study was further confirmed based on preliminary vaccination results in pigs with 7 mutants (n=8) described below in Example 11.

### Example 10 Characterization of Attenuated A. pleuropneumoniae Virulence Genes

The A. pleuropneumoniae genes identified represent four broad functional classes: biosynthetic enzymes, cellular transport components, cellular regulation components and unknowns.

The atpG gene, encoding the F1- $\gamma$  subunit of the F<sub>0</sub>F<sub>1</sub> H+-ATPase complex, can function in production of ATP or in the transport of protons by hydrolyzing ATP. A related atpG attenuated mutant was also identified in P. multocida. Another atp gene, atpH, that encodes the F<sub>1</sub>  $\delta$  subunit was also identified. Phenotypes of atp mutants include non-adaptable acid-sensitivity phenotype [Foster, J Bacteriol. 173:6896-6902 (1991)], loss of virulence in Salmonella typhimurium [Garcia del Portillo, et al., Infect Immun. 61:4489-4492 (1993)] and P. multocida (above) and a reduction in both transformation frequencies and induction of competence regulatory genes in Haemophilus influenzae Rd [Gwinn, et al., J Bacteriol. 179:7315-20 (1997)].

LpdA is a dihydrolipoamide dehydrogenase that is a component of two enzymatic complexes: pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase.

While the relationship to virulence is unknown, production of LpdA is induced in *Salmonella typhimurium* when exposed to a bactericidal protein from human which may suggest that this induction may be involved in attempts to repair the outer membrane [Qi, et al., Mol Microbiol. 17:523-31 (1995)].

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Transport of scarce compounds necessary for growth and survival are critical in vivo. ExbB is a part of the TonB transport complex [Hantke, and Zimmerman, Microbiology Letters. 49:31-35 (1981)], interacting with TonB in at least two distinct ways [Karlsson, et al., Mol Microbiol. 8:389-96 (1993), Karlsson, et al., Mol Microbiol. 8:379-88 (1993)]. Iron acquisition is essential for pathogens. In this work, attenuated exbB mutants in both APP and P. multocida have been identified. Several TonBdependent iron receptors have been identified in other bacteria [Biswas, et al., Mol. Microbiol. 24:169-179 (1997), Braun, FEMS Microbiol Rev. 16:295-307 (1995), Elkins, et al., Infect Immun. 66:151-160 (1998), Occhino, et al., Mol Microbiol. 29:1493-507 (1998), Stojiljkovic and Srinivasan, *J Bacteriol*. 179:805-12 (1997)]. pleuropneumoniae produces 2 transferrin-binding proteins, which likely depend on the ExbB/ExbD/TonB system, for acquisition of iron. PotD is a periplasmic binding protein that is required for spermidine (a polyamine) transport [Kashiwagi, et al., J Biol Chem. 268:19358-63 (1993)]. Another member of the Pasteurellaceae family, Pasteurella haemolytica, contains a homologue of potD (Lpp38) that is a major immunogen in convalescent or outer membrane protein vaccinated calves [Pandher and Murphy, Vet Microbiol. 51:331-41 (1996)]. In P. haemolytica, PotD appeared to be associated with both the inner and outer membranes. The role of PotD in virulence or in relationship to protective antibodies is unknown although previous work has shown potD mutants of Streptococcus pneumoniae to be attenuated [Polissi, et al., Infect. Immun. 66:5620-9 (1998)].

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Relatively few "classical virulence factors," such as adhesins or toxins with the exception of homologues to OMP P5 of *Haemophilus influenzae*, were identified. *H. influenzae* OMP P5 is a major outer membrane protein that is related to the OmpA porin family of proteins [Munson, *et al.*, *M Infect Immun. 61*:4017-20(1993)]. OMP P5 in nontypeable *Haemophilus influenzae* has been shown to encode a fimbrial subunit protein expressed as a filamentous structure [Sirakova, *et al.*, *Infect Immun.* 

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62:2002-20 (1994)] that contributes to virulence and binding of both mucin and epithelial cells [Miyamoto and Bakaletz, *Microb Pathog. 21*:343-56 (1996), Reddy, *et al.*, *Infect Immun. 64*:1477-9 (1996), Sirakova, *et al.*, *Infect Immun. 62*:2002-20 (1994)]. A significant finding was identification of two distinct ORF's that appear to encode OMP P5 homologues. This is also the case with two very similar proteins, MOMP and OmpA2 from *Haemophilus ducreyi*. It remains to be determined whether both are functionally involved in the production of fimbriae and whether the presence of two such ORFs represents a divergent duplication with redundant or complementing functions. Interestingly, the two OMP P5 mutants seem to have disparate CI values, suggesting a difference in essentiality or functionality for only one copy. OMP P5 has been shown to undergo molecular variation during chronic infections [Duim, *et al.*, *Infect Immun. 65*:1351-1356 (1997)], however, this appears to be restricted to a single gene undergoing point mutations resulting in amino acid changes rather than "type switching" due to differential expression of multiple genes.

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Protein folding enzymes are important accessories for the efficient folding of periplasmic and extracellular proteins, and two genes were identified whose products have peptidyl-prolyl isomerase activity: fkpA and tig (trigger factor). FkpA is a periplasmic protein that is a member of the FK506-binding protein family [Horne and Young, Arch Microbiol. 163:357-65 (1995); Missiakas, et al., Mol Microbiol. 21:871-84 (1996)]. FkpA has been shown to contribute to intracellular survival of Salmonella typhimurium [Horne, et al., Infect Immun. 65:806-10 (1997)] and a Legionella pneumophila homolog, mip [Engleberg, et al., Infect Immun. 57:1263-1270 (1989)], is responsible for virulence and infection of macrophages [Cianciotto, et al., J. Infect. Dis. 162:121-6 (1990); Cianciotto, et al., Infect. Immun. 57:1255-1262 (1989)]. Tig, or trigger factor [Crooke and Wickner, Proc. Natl. Acad. Sci. USA. 84:5216-20 (1987), Guthrie, and Wickner, J Bacteriol. 172:5555-62 (1990), reviewed in Hesterkamp, and Bukau., FEBS Lett. 389:32-4 (1996)], is a peptidyl prolyl isomerase containing a typical FKBP region [Callebaut and Mornon, FEBS Lett. 374:211-215 (1995)], but is unaffected by FK506 [Stoller, et al., EMBO J. 14:4939-48 (1995)]. Tig has been shown to associate with the ribosomes and nascent polypeptide chains [Hesterkamp, et al., Proc Natl Acad Sci USA 93:4437-41 (1996), Stoller, et al., EMBO J. 14:4939-48 (1995)]. Possible roles

include an unknown influence on cell division [Guthrie, and Wickner, *J Bacteriol.* 172:5555-62 (1990)] in *E. coli*, a role in the secretion and activation of the *Streptococcus pyogenes* cysteine proteinase [Lyon, *et al.*, *EMBO J.* 17:6263-75 (1998)] and survival under starvation conditions in *Bacillus subtilis* [Gothel, *et al.*, *Biochemistry* 37:13392-9 (1998)].

Bacterial pathogens employ many mechanisms to coordinately regulate gene expression in order to survive a wide variety of environmental conditions within the host. Differences in mRNA stability can modulate gene expression in prokaryotes [Belasco and Higgins, *Gene 72*:15-23 (1988)]. For example, *rnr* (*vacB*) is required for expression of plasmid borne virulence genes in *Shigella flexneri* [Tobe, *et al.*, *J. Bacteriol. 174*:6359-67 (1992)] and encodes the RnaseR ribonuclease [Cheng, *et al.*, *J. Biol. Chem. 273*:14077-14080 (1998)]. PNP is a polynucleotide phosphorylase that is involved in the degradation of mRNA. Null *pnp / rnr* mutants are lethal, suggesting a probable overlap of function. It therefore is possible that both *rnr* and *pnp* are involved in the regulation of virulence gene expression. A *pnp* mutant of *P. multocida* is avirulent in a mouse septicemic model (Example 2)]. Other *pnp*-associated phenotypes include competence deficiency and cold sensitivity in *Bacillus subtilis* [Wang and Bechhofer, *J. Bacteriol. 178*:2375-82 (1996)].

HupA is a bacterial histone-like protein, which in combination with HupB constitute the HU protein in E. coli. Reports have suggested that *hupA* and *hupB* single mutants do not demonstrate any observable phenotype [Huisman, *et al.*, *J Bacteriol*. 171:3704-12 (1989), Wada, *et al.*, *J Mol Biol*. 204:581-91 (1988)], however, *hupA-hupB* double mutants have been shown to be cold sensitive, sensitive to heat shock and blocked in many forms of site-specific DNA recombination [Wada, *et al.*, *J Mol Biol*. 204:581-91 (1988), Wada, *et al.*, *Gene*. 76:345-52 (1989)]. One limited data previously indicated that *hupA* is directly involved in virulence [Turner, *et al.*, *Infect Immun*. 66:2099-106 (1998)]. The mechanism of *hupA* attenuation remains unknown.

DnaK is a well known and highly conserved heat shock protein involved in regulatory responses to various stressful environmental changes [reviewed in Lindquist and Craig, *Annu Rev Genet. 22*:631-77 (1988)]. DnaK is also one of the most significantly induced stress proteins in *Yersinia enterocolitica* after being phagocytosed

by macrophages [Yamamoto, et al., Microbiol Immunol. 38:295-300 (1994)] and a Brucella suis dnaK mutant failed to multiply within human macrophage-like cells [Kohler, et al., Mol Microbiol. 20:701-12 (1996)]. In contrast, another intracellular pathogen, Listeria monocytogenes, did not show induction of dnaK after phagocytosis [Hanawa, et al., Infect Immun. 63:4595-9 (1995)]. A dnaK mutant of Vibrio cholera affected the production of ToxR and its regulated virulence factors in vitro but similar results were not obtained from in vivo grown cells [Chakrabarti, et al., Infect Immun. 67:1025-1033 (1999)]. The CI of A. pleuropneumonia dnaK mutant was higher than most of the attenuated mutants although still approximately half of the positive control strain.

DksA is a dosage dependent suppressor of filamentous and temperature-sensitive growth in a *dnaK* mutant of *E. coli* [Kang and Craig, *J Bacteriol. 172*:2055-64 (1990)]. There is currently no defined molecular function for DksA, but the gene has been identified as being critical for the virulence of *Salmonella typhimurium* in chickens and newly hatched chicks [Turner, *et al.*, *Infect Immun. 66*:2099-106 (1998)]. In that work, it was noted that the *dksA* mutant did not grow well with glucose or histidine but did grow well with glutamine or glutamate as the sole carbon source. This observation may indicate that the *dksA* mutant is somehow impaired in the biosynthesis of glutamate [Turner, *et al.*, *Infect Immun. 66*:2099-106 (1998)].

Three genes were identified that have roles in protein synthesis: tRNA-leu, tRNA-glu and rpmF. Excluding protein synthesis, tRNA's also have a wide variety of functional roles in peptidoglycan synthesis [Stewart, et al., Nature 230:36-38 (1971)], porphyrin ring synthesis [Jahn, et al., Trends Biochem Sci. 17:215-8 (1992)], targeting of proteins for degradation [Tobias, et al., Science 254:1374-7 (1991)], post-translational addition of amino acids to proteins [Leibowitz and Soffer, B.B.R.C. 36:47-53 (1969)] and mediation of bacterial-eukaryotic interactions [Gray, et al., J Bacteriol. 174:1086-98 (1992), Hromockyj, et al., Mol Microbiol. 6:2113-24 (1992)]. More specifically, tRNA-leu is implicated in transcription attenuation [Carter, et al., Proc. Natl. Acad. Sci. USA 83:8127-8131 (1986)], lesion formation by Pseudomonas syringae [Rich and Willis, J Bacteriol. 179:2247-58 (1997)] and virulence of uropathogenic E. coli [Dobrindt, et al., FEMS Microbiol Lett. 162:135-141 (1998), Ritter, et al., Mol Microbiol. 17:109-21

(1995)]. It is unknown whether the tRNA that we have identified represents a minor species of tRNA-leu in *A. pleuropneumoniae*. Regardless, it is possible that tRNA-leu may have any one of a wide range of functions. RpmF is a ribosomal protein whose gene is also part of an operon containing fatty acid biosynthesis enzymes in *E. coli*. Further work will be required to indicate if this is the case in *A. pleuropneumoniae*, although the same clustering of *fab* genes and *rpmF* occurs in *Haemophilus influenzae* [Fleischmann, *et al.*, *Science 269*:496-512 (1995)]. The expression of the *fab* genes is not necessarily dependent on transcripts originating upstream of *rpmF* as there has been a secondary promoter identified within *rpmF* [Zhang and Cronan, Jr., *J Bacteriol. 180*:3295-303 (1998)].

The final class of attenuated mutants includes mutations within genes of unknown function or genes that have not been previously identified. Homologs of *yaeA* and HI0379 have previously been identified in *Escherichia coli* [Blattner, *et al.*, *Science* 277:1453-1474 (1997)] and *Haemophilus influenzae* [Fleischmann, *et al.*, *Science* 269:496-512 (1995)], respectively. The remaining unknowns have been designated Actinobacillus pleuropneumoniae virulence genes (apv). The *apvC* gene shows significant similarity to HI0893, however, the proposed similarity of HI0893 as a transcriptional repressor similar to the fatty acid response regulator Bm3R1 [Palmer, *J Biol Chem.* 273:18109-16 (1998)] is doubtful. The *apvD* gene is also most similar to a putative membrane protein (b0878) with unknown function from *E. coli* [Blattner, *et al.*, *Science* 277:1453-1474 (1997)]. Two other unknowns, *apvA* and *apvB* had no significant matches in the public databases.

### Example 11 Safety and Efficacy of *A. pleuropneumoniae* Mutants

Nine groups (n=8) of SPF pigs (4-5 weeks old, 3-10 kg) were used to determine the safety and efficacy of seven *A. pleuropneumoniae* mutants as live attenuated vaccine strains. Seven groups were infected intranasally with  $10^{10}$  CFU of each mutant on day 1. One group was vaccinated on days 1 and 15 with the commercially available vaccine Pleuromune (Bayer), and one naive group was not vaccinated. On day 29, all groups were challenged intranaslally with 1-5 x  $10^5$  CFU per

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pig of wild type APP225. All surviving animals were euthanized and necropsied on day 42 of the study. Results are shown in Table 4.

Table 4
Efficacy of A. pleuropneumoniae Mutants

Vaccine	% Mortality following intranasal challenge						
	<b>Vaccination</b>	<b>Challenge</b>					
Pleuromune	0	37.5					
exbB	0	0					
tig	12.5	0					
fkpA	12.5	0					
HI0385	50.0	0					
pnp	0	0					
yaeE	0	0					
atpG	0	0					
None	N/A	50.0					

The exbB, atpG, pnp, and yaeA mutants caused no mortality when administered at a dosage of  $10^{10}$  CFU intranasally. The fkpA and tig mutant groups had one death each and the HI0379 group (highest April 6, 2000CI of the 7 mutants tested shown in Example 9) had four deaths. Wildtype  $LD_{50}$  using this model was generally 1 x  $10^7$  CFU, indicating that each of these mutants is at least 100 fold attenuated and that there is a reasonable correlation between CI and attenuation.

Numerous modifications and variations in the invention as set forth in the above illustrative examples are expected to occur to those skilled in the art. Consequently only such limitations as appear in the appended claims should be placed on the invention.

#### WHAT IS CLAIMED IS:

1. A gram-negative bacteria comprising a mutation in a gene represented by a nucleotide sequence set forth in any one of SEQ ID NOs: 1, 3, 7, 9, 21, 25, 27, 29, 39, 41, 51, 53, 55, 57, 58, 60, 68, 72, 74, 76, 78, 80, 82, 84, 104, 108, 112, 116, 118, 120 122, 124, 126, 128, and 130, or species homologs thereof, said mutation resulting in decreased activity of a gene product encoded by the mutated gene.

- 2. The gram-negative bacteria of claim 1 wherein said mutation results in decreased expression of a gene product encoded by the mutated gene.
- 3. The gram-negative bacteria of claim 1 wherein said mutation results in expression of an inactive gene product encoded by the mutated gene.
- 4. The gram-negative bacteria of claim 1 wherein said mutation results in deletion of all or part of said gene.
- 5. The gram-negative bacteria of claim 1 wherein said mutation results in deletion of at least about 10%, at least about 20%, at least about 30%, at least about 40% at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98%, or at least about 99% of said gene.
- 6. The gram-negative bacteria of claim 1 wherein said mutation results in an insertion in the gene, said insertion causing decreased expression of a gene product encoded by the mutated gene and/or expression of an inactive gene product encoded by the mutated gene.

An attenuated *Pasteurellaceae* bacteria comprising a mutation in a gene represented by a nucleotide sequence set forth in any one of SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124,

126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, and 164, or a species homolog thereof, said mutation resulting in decreased activity of a gene product encoded by the mutated gene.

- 8. The *Pasteurellaceae* bacteria of claim 7 wherein said mutation results in decreased expression of a gene product encoded by the mutated gene.
- 9. The *Pasteurellaceae* bacteria of claim 7 wherein said mutation results in expression of an inactive gene product encoded by the mutated gene.
- 10. The *Pasteurellaceae* bacteria of claim 7 wherein said mutation results in deletion of all or part of said gene.
- 11. The *Pasteurellaceae* bacteria of claim 7 wherein said mutation results in deletion of at least about 10%, at least about 20%, at least about 30%, at least about 40% at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98%, or at least about 99% of said gene.
- 12. The *Pasteurellaceae* bacteria of claim 7 wherein said mutation results in an insertion in the gene, said insertion causing decreased expression of a gene product encoded by the mutated gene and/or expression of an inactive gene product encoded by the mutated gene.
- 13. The *Pasteurellaceae* bacteria of claim 7 selected from the group consisting of *Pasteurella haemolytica*, *Pasteurella multocida*, *Actinobacillus pleuropneumoniae* and *Haemophilus somnus*.
- 14. The *Pasteurellaceae* bacteria of claim 13 wherein said mutation results in decreased expression of a gene product encoded by the mutated gene.

- 15. The *Pasteurellaceae* bacteria of claim 13 wherein said mutation results in expression of an inactive gene product encoded by the mutated gene.
- 16. The *Pasteurellaceae* bacteria of claim 13 wherein said mutation results in deletion of all or part of said gene.
- 17. The *Pasteurellaceae* bacteria of claim 13 wherein said mutation results in deletion of at least about 10%, at least about 20%, at least about 30%, at least about 40% at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98%, or at least about 99% of said gene.
- 18. The *Pasteurellaceae* bacteria of claim 13 wherein said mutation results in an insertion in the gene, said insertion causing decreased expression of a gene product encoded by the mutated gene and/or expression of an inactive gene product encoded by the mutated gene.
- 19. The attenuated *Pasteurellaceae* bacteria of claim 13 that is a *P. multocida* bacteria.
- 20. The *Pasteurellaceae* bacteria of claim 19 wherein said mutation results in decreased expression of a gene product encoded by the mutated gene.
- 21. The *Pasteurellaceae* bacteria of claim 19 wherein said mutation results in expression of an inactive gene product encoded by the mutated gene.
- 22. The *Pasteurellaceae* bacteria of claim 19 wherein said mutation results in deletion of all or part of said gene.
- 23. The *Pasteurellaceae* bacteria of claim 19 wherein said mutation results in deletion of at least about 10%, at least about 20%, at least about 30%, at least

about 40% at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98%, or at least about 99% of said gene.

- 24. The *Pasteurellaceae* bacteria of claim 19 wherein said mutation results in an insertion in the gene, said insertion causing decreased expression of a gene product encoded by the mutated gene and/or expression of an inactive gene product encoded by the mutated gene.
- 25. The attenuated *Pasteurellaceae* bacteria of claim 13 that is a *A. pleuropneumoniae* bacteria.
- 26. The *Pasteurellaceae* bacteria of claim 25 wherein said mutation results in decreased expression of a gene product encoded by the mutated gene.
- 27. The *Pasteurellaceae* bacteria of claim 25 wherein said mutation results in expression of an inactive gene product encoded by the mutated gene.
- 28. The *Pasteurellaceae* bacteria of claim 25 wherein said mutation results in deletion of all or part of said gene.
- 29. The *Pasteurellaceae* bacteria of claim 25 wherein said mutation results in deletion of at least about 10%, at least about 20%, at least about 30%, at least about 40% at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98%, or at least about 99% of said gene.
- 30. The *Pasteurellaceae* bacteria of claim 25 wherein said mutation results in an insertion in the gene, said insertion causing decreased expression of a gene product encoded by the mutated gene and/or expression of an inactive gene product encoded by the mutated gene.

- 31. An immunogenic composition comprising the bacteria according to any one of claims 1 through 30.
- 32. A vaccine composition comprising the immunogenic composition according to claim 31 and a pharmaceutically acceptable carrier.
- 33. The vaccine composition according to claim 32 further comprising an adjuvant.

34. A method for producing a gram-negative bacteria mutant comprising the step of introducing a mutation in a gene represented by a nucleotide sequence set forth in any one of SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, and 164, or a species homolog thereof, said mutation resulting in decreased activity of a gene product encoded by the mutated gene.

35. A method for producing an attenuated *Pasteurellaceae* bacteria comprising the step of introducing a mutation in a gene represented by a nucleotide sequence set forth in any one of SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, and 164, or a species homolog thereof, said mutation resulting in decreased activity of a gene product encoded by the mutated gene.

36. A purified and isolated *Pasteurellaceae* polynucleotide comprising a nucleotide sequence selected from the group consisting of nucleotide sequences set forth in SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112,

114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, and 164, .

37. A purified and isolated *Pasteurellaceae* polynucleotide comprising a nucleotide sequence selected from the group consisting of nucleotide sequences set forth in SEQ ID NOs: 1, 3, 7, 9, 21, 25, 27, 29, 39, 41, 51, 53, 55, 57, 58, 60, 68, 72, 74, 76, 78, 80, 82, 84, 104, 108, 112, 116, 118, 120 122, 124, 126, 128, and 130.

- 38. A purified and isolated polynucleotide encoding a *Pasteurellaceae*. virulence gene product, or species homolog thereof, selected from the group consisting of:
  - a) the polynucleotide according to claim 37,
  - b) polynucleotides encoding a polypeptide encoded by the polynucleotide of (a), and
  - c) polynucleotides that hybridize to the complement of the polynucleotides of (a) or (b) under moderate stringency conditions.
- 39. A purified and isolated *Pasteurellaceae* polynucleotide encoding a polypeptide selected from the group consisting of polypeptides having amino acid sequences set forth in SEQ ID NOs: 2, 4, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 30, 32, 34, 38, 40, 42, 52, 54, 56, 59, 61, 69, 71, 73, 75, 77, 79, 81, 83, 85, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, and 165.
  - 40. The polynucleotide of claim 39 which is a DNA.
  - 41. A vector comprising the DNA of claim 40.
- 42. The vector of claim 41 that is an expression vector, wherein the DNA is operatively linked to an expression control DNA sequence.

- 43. A host cell stably transformed or transfected with the DNA of claim 40 in a manner allowing the expression of the encoded polypeptide in said host cell.
- 44. A method for producing a recombinant polypeptide comprising culturing the host cell of claim 43 in a nutrient medium and isolating the encoded polypeptide from said host cell or said nutrient medium.
  - 45. A purified polypeptide produced by the method of claim 44.
- 46. A purified polypeptide comprising a polypeptide selected from the group consisting of polypeptides having amino acid sequences set forth in SEQ ID NOs: 2, 4, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 30, 32, 34, 38, 40, 42, 52, 54, 56, 59, 61, 69, 71, 73, 75, 77, 79, 81, 83, 85, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, and 165.
- 47. An antibody that is specifically reactive with the polypeptide of claim 46.
  - 48. The antibody of claim 47 that is a monoclonal antibody.
- 49. A method of using the monoclonal antibody of claim 39 for identifying a bacteria of claim 1, 7, 13, or 19 comprising the step of contacting an extract of bacteria with said monoclonal antibody and detecting the absence of binding of said monoclonal antibody.
- 50. A method of identifying an anti-bacterial agent comprising the steps of assaying potential agents for the ability to interfere with expression or activity of gene products represented by the amino acid sequences set forth in any one of SEQ ID NOS: 2, 4, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 30, 32, 34, 38, 40, 42, 52, 54, 56, 59, 61, 69, 71, 73, 75, 77, 79, 81, 83, 85, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123,

125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, and 165 and identifying an agent that interferes with expression or activity of said gene products.

of:

51. A method of identifying an anti-bacterial agent comprising the steps

- a) measuring expression or activity of a gene product as set out in SEQ ID NOs: 2, 4, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 30, 32, 34, 38, 40, 42, 52, 54, 56, 59, 61, 69, 71, 73, 75, 77, 79, 81, 83, 85, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, and 165 b) contacting the gene product in (a) with a test compound
- c) measuring expression or activity of the gene product in the presence of the test compound; and
- d) identifying the test compound as an antibacterial agent when expression or activity of the gene product is decreased in the presence of the test compound as compared to expression or activity in the presence of the test compound.

### **ABSTRACT**

Gram negative bacterial virulence genes are identified, thereby allowing the identification of novel anti-bacterial agents that target these virulence genes and their products, and the provision of novel gram negative bacterial mutants useful in vaccines.

#### SEQUENCE LISTING

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Trp His Val His Leu Asp Thr Leu Leu Phe Ser Ile Ile Ser Gly Ala
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<sup>&</sup>lt;210> 2

<sup>&</sup>lt;211> 264

<sup>&</sup>lt;212> PRT

<sup>&</sup>lt;213> Pasteurella multocida

<sup>&</sup>lt;400> 2

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100

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Ala Ile Gly Leu Lys Asn Ser Lys Ile Ser Asn Gly Asp Leu Glu Phe

Ile Val Leu Trp Gly Arg Thr Arg Asp Leu Phe Val Asn Gly Glu Lys

Pro Thr Arg Phe Arg Asp Lys Thr Met Leu Ser Val Leu Pro Asn Leu

Ile Gly Asn Arg Leu Ser Ile His Asp Ile Asp Gln Leu Ile Glu Ile

Leu Asn Thr Thr Asn Lys Lys Ala Thr Val Asn Val Val Ala Ser Glu 120

450

Glu Lys Gly Ser Ser Asn Leu Asn Ile Glu Arg Gln Tyr Asp Val Phe Pro Gln Val Ser Val Gly Phe Asn Asn Ser Gly Ala Gly Asn Asn Ala 150 Asn Gly Arg Asn Gln Ala Thr Leu Asn Ile Ala Trp Ser Asp Leu Leu 170 Gly Thr Asn Asp Arg Trp Ser Phe Ser Ser Ser Tyr Arg Leu Tyr Lys 185 180 Asn His His Ala Asn Gln Gln Arg Asn Tyr Thr Leu Ser Tyr Ser Gln Pro Ile Gly Phe Ser Thr Val Glu Ile Lys Ala Ser Glu Ser Thr Tyr 215 Glu Lys Glu Leu Arg Gly Ile Asn Thr His Ser Ser His Gly Lys Thr 230 Gln Ser Leu Ala Val Lys Leu Met His Val Leu Leu Arg Asn Lys Glu Ser Ile Leu Ser Thr Tyr Thr Glu Phe Glu Phe Lys Lys Arg Ile Ser Tyr Phe Ser Asp Ile Leu Ile Gly Lys Tyr His Asn Asn Lys Val Ser 280 Val Gly Leu Ser Tyr Met Thr Asn Phe Ala Tyr Gly Lys Leu Tyr Ser Asp Ile Ala Tyr Ala Asn Gly Leu Arg Trp Phe Gly Ala Asn Tyr Ser 305 Ala Tyr Asp Ala Asn Arg Glu Lys Thr Leu Lys Leu Leu Ser Gly Ser 330 Ile Asn Trp Gln Arg Pro Ile Ser Leu Phe Glu Arg Ala Met Asn Tyr 345 Gln Leu Arg Ile Gly Ala Gln Tyr Gly Phe Asp Ser Leu Tyr Ser Glu Asn Gln Phe Ser Ile Gly Asp Glu Tyr Thr Val Arg Gly Phe Lys Gly Gly Ala Val Ser Gly Asp Ser Gly Ala Tyr Leu Ser Gln Thr Leu Thr 390 Val Pro Phe Tyr Pro Gln Lys Ala Tyr Leu Ser Gln Val Ser Pro Phe 410 Ile Gly Phe Asp Met Gly Lys Val His Ile Lys Ser Lys His Lys Thr Thr Thr Leu Val Gly Phe Ala Leu Gly Leu Lys Thr Gln Ile Lys Leu 440 Phe Ser Leu Ser Leu Thr Tyr Ala Gln Pro Met Asn Gly Val Ser Gly

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Val Thr Gln His Arg Gln Lys Pro Ile Tyr Tyr Phe Ser Gly Ser Leu

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ct Le	u II	t Le 20	tca Ser	gtt Val	aac Asn	gcc Ala	acg Thr 125	cga Arg	ttg Leu	aat Asn	taga	agaaa	agc t	aaat	gga	tt	1042
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I	e A	la	Glu 35	Ala	Arg	Glu	His	Gly 40		Leu	Lys	Glu	Asn 45	Ala	Glu	Tyr	
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P	co A	.sn	Asn	Gly	Lys 85		Ile	Phe	Gly	Ala 90	Thr	Ile	Leu	Leu	Leu 95	ı Asn	
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95 100 105

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<sup>&</sup>lt;210> 24

<sup>&</sup>lt;211> 487

<sup>&</sup>lt;212> PRT

<sup>&</sup>lt;213> Pasteurella multocida

<sup>&</sup>lt;400> 24

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Val Pro Ala His Ser Thr Val Leu Pro Asn Thr Ala Asp Leu Ser Thr
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<sup>&</sup>lt;212> PRT

<sup>&</sup>lt;213> Pasteurella multocida

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His	Leu	Leu	Thr	Leu 245	Ser	Ser	Val	Tyr	Gly 250	Ile	His	Lys	Gly	Trp 255	Glu
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Tyr His Lys Gly Gly Val Lys Lys Ala Asp Tyr Asn Tyr Gly Tyr Phe 385 390 395 400

Gln Pro Tyr Tyr Met Pro Ser Gly Arg Gln Tyr Thr Gln Ala Phe Tyr 405 410 415

Leu Gln Asp Gln Ile Lys Trp Gln Asn Phe Leu Phe Thr Gly Gly Ile 420 425 430

Arg Tyr Asp His Ile Asn Asn Ile Gly Gln Lys Asn Leu Ala Pro Arg 435 440 445

Tyr Asn Asp Ile Ser Ala Gly His Asp Tyr Ser Gln Lys Asn Tyr Asn 450 455 460

Gly Trp Ser Tyr Tyr Leu Gly Leu Lys Tyr Asp Val Asn His Tyr Leu 465 470 475 480

Ser Leu Phe Thr Asn Phe Ser Lys Thr Trp Arg Ala Pro Val Ile Asp 485 490 495

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Leu Asp Thr Ile Thr Val Ser Ser Gln Gln Asp Glu Met Asn Ile Lys
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Gln Gln Val Gln Asp Ser Arg Asp Leu Val Arg Tyr Glu Thr Gly Val 65 70 75 80

Thr Val Val Glu Ala Gly Arg Phe Gly Ser Ser Gly Tyr Ala Ile Arg 85 90 95

Gly Val Asp Glu Asn Arg Val Ala Ile Thr Val Asp Gly Leu His Gln

100 105 110

Ala Glu Thr Leu Ser Ser Gln Gly Phe Lys Glu Leu Phe Glu Gly Tyr 120 Gly Asn Phe Asn Asn Thr Arg Asn Ser Val Glu Ile Glu Thr Leu Lys Val Ala Lys Ile Ala Lys Gly Ala Asp Ser Val Lys Val Gly Ser Gly Ser Leu Gly Gly Ala Val Leu Phe Glu Thr Lys Asp Ala Arg Asp Phe 170 Leu Thr Glu Lys Asp Trp His Ile Gly Tyr Lys Ala Gly Tyr Ser Thr Ala Asp Asn Gln Gly Leu Asn Ala Val Thr Leu Ala Gly Arg Tyr Gln 200 Met Phe Asp Ala Leu Ile Met His Ser Lys Arg His Gly His Glu Leu Glu Asn Tyr Asp Tyr Lys Asn Gly Arg Asp Ile Gln Gly Lys Glu Arg 225 Glu Lys Ala Asp Pro Tyr Thr Ile Thr Lys Glu Ser Thr Leu Val Lys 250 Phe Ser Phe Ser Pro Thr Glu Asn His Arg Phe Thr Val Ala Ser Asp Thr Tyr Leu Gln His Ser Arg Gly His Asp Leu Ser Tyr Asn Leu Val 280 Ala Thr Thr His Ile Gln Leu Asp Glu Lys Glu Ser Arg His Ala Asn Asp Leu Thr Lys Arg Lys Asn Val Ser Phe Thr Tyr Glu Asn Tyr Thr Val Thr Pro Phe Trp Asp Thr Leu Lys Leu Ser Tyr Ser Gln Gln Arg 330 Ile Thr Thr Arg Ala Arg Thr Glu Asp Tyr Cys Asp Gly Asn Glu Leu Cys Asp Ser Tyr Lys Asn Pro Leu Gly Leu Gln Phe Lys Asp Gly Gln Ile Leu Asp Pro Ala Gly Asn Lys Ile Lys Leu Gln Gly Ser Gly Leu Ser Thr Gln Ile Val Asp Glu Asn Gly Lys Pro Phe Pro Thr Thr Gly Thr Asn Asn Ala Ala Phe Ser Asn Asn Leu Arg Leu Arg Pro Thr 410 Gly Phe Trp Leu Asp Cys Ser Val Phe Asp Cys Asn Lys Pro Phe Thr 425

Val Tyr Asn Ile Ser Asn Gly Thr Tyr Gln Ala Arg Glu Val Leu Leu 435 Ser Glu Glu Ile Thr Val Asp Gly Lys Leu Tyr Lys Thr Ala Lys Glu Glu Gly Gly Leu Pro Asn Tyr Leu Ile Leu Pro Asn Ser Lys Gly Tyr Leu Pro Tyr Asp Tyr Lys Glu Arg Asp Leu Asn Thr Asn Thr Lys Gln 490 Ile Asn Leu Asp Leu Thr Lys Thr Phe Leu Thr Phe Asn Ile Glu Asn 505 Asn Leu Ser Tyr Gly Gly Val Tyr Ser Arg Ile Glu Lys Glu Met Ile Asn Lys Ala Gly Tyr Glu Gly Arg Asn Pro Thr Trp Trp Ala Asp Arg Ile Leu Gly Gln Ser Ser Tyr Cys Gly Tyr Asn Ala Leu Lys Cys Pro 555 Lys His Glu Pro Leu Thr Ser Phe Leu Ile Pro Val Glu Ala Thr Thr 570 Gln Ser Leu Tyr Phe Ala Asn Ile Leu Lys Val His Asn Met Ile Ser Ile Asp Leu Gly Tyr Arg Tyr Asp His Ile Lys Tyr Asn Pro Glu Tyr Thr Pro Gly Val Thr Pro Lys Ile Pro Asp Asp Met Val Lys Gly Leu Phe Ile Pro Met Pro Lys Glu Pro Gln Leu Lys Asp Phe Asp Tyr Asn Tyr Ala Lys Phe Gly Glu Ala Tyr Lys Lys Trp Lys Glu Tyr Leu Pro Lys Asn Ala Glu Glu Asn Ile Ala Tyr Ile Ala Gln Asp Lys Thr Phe 660 Lys Lys His Ser Tyr Ser Leu Gly Ala Thr Phe Asp Pro Leu Asn Phe Leu Arg Val Gln Val Lys Tyr Ser Lys Gly Phe Arg Ala Pro Thr Ser Asp Glu Leu Tyr Phe Thr Phe Lys His Pro Asp Phe Thr Ile Leu Pro 715 Asn Pro Val Leu Lys Pro Glu Glu Ala Lys Asn Gln Glu Ile Ala Leu Thr Val His Asp Asn Trp Gly Phe Val Ser Thr Ser Val Phe Gln Thr

765

Lys Tyr Arg His Phe Ile Asp Leu Ala Tyr Leu Gly Ser Arg Asn Leu 760

Ser Asn Ser Val Gly Gly Gln Ala Gln Ala Arg Asp Phe Gln Val Tyr 770 775 780

Gln Asn Val Asn Val Asp Asn Ala Lys Val Lys Gly Leu Glu Ile Asn 785 790 795 800

Ala Arg Leu Asn Leu Gly Tyr Phe Trp His Val Leu Asp Gly Phe Asn 805 810 815

Thr Ser Tyr Lys Phe Thr Tyr Gln Arg Gly Arg Leu Asp Gly Asp Arg 820 825 830

Pro Met Asn Ala Ile Gln Pro Lys Ala Ser Val Phe Gly Leu Gly Tyr 835 840 845

Asp His Lys Glu Asn Lys Phe Gly Ala Asp Leu Tyr Ile Thr Arg Val 850 855 860

Ser Glu Lys Lys Ala Lys Asp Thr Tyr Asn Met Phe Tyr Lys Glu Gln 865 870 875 880

Gly Tyr Lys Asp Ser Ala Val Arg Trp Arg Ser Asp Asp Tyr Thr Leu 885 890 895

Val Asp Ala Val Gly Tyr Ile Lys Pro Ile Lys Asn Leu Thr Leu Gln 900 905 910

Phe Gly Val Tyr Asn Leu Thr Asp Arg Lys Tyr Leu Thr Trp Glu Ser 915 920 925

Ala Arg Ser Ile Lys Pro Phe Gly Thr Ser Asn Leu Ile Asn Gln Lys 930 935 940

Thr Gly Ala Gly Ile Asn Arg Phe Tyr Ser Pro Gly Arg Asn Phe Lys 945 950 955 960

Leu Ser Ala Glu Ile Thr Phe 965

<210> 33

<211> 2990

<212> DNA

<213> Pasteurella multocida

<220>

<221> CDS

<222> (1106) .. (1564)

<220>

<223> kdtB

<400> 33

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tgccccttct ttcggtgctt ttgaggagcg cacatgggct aaaaagcgtg tgtcggataa 360 aatccqctcq accaacactt ccaccttacc gccactggct ttacgtccaa acatccttgc 420 aggaatcacg cgcgtgttat taaaaattaa taagtcgcct tcatgaattt gatcaaggat 480 atcagcaaaa gtgcggtggg taatctcacc attttcgccg ttaagttgta ataagcgact 540 agcggtgcga tccggttttg ggtaacgagc aatcagctca tcgggtaaat caaaataaaa 600 qtcaqaaaca cqcataaata gggttataaa aagttatcta aaaaatcgtg ggcgtaagtc 660 tagtqtgaat teegetettg cacaaggaaa aatecagatt ttgttgttta gtategaatt 720 gagatgattt tggacaaaaa aaaagccctt tcaagaaaga cgaaagggcg aaaatatatt 780 tggagtcata ctttttaggg tatgtgtcgg attatacaca caaaaataac aaatgcaaca 840 tttttttaac aatcatatgt aagcgtattg tgtgagaacg agcgtaaaaa tgaacgcatt 900 ctaaaqqatq atttatttag cctattaaaa aaacacatga gatgagagtt tgcgagagcg 960 gtaataaaag tgcggtgggt tttagaaaag ttttgaatag gatcacaaat taaacaaagt 1020 ttgtgaaata ccaagtagta gtttttaagt atatgatgaa tcatatgcta aagtttaaac 1080 ccqttaaata accaagaggt ggaag atg aca gaa gaa aat aaa gga aag aga Met Thr Glu Glu Asn Lys Gly Lys Arg tat ttt tta tqq ttc ata ttq ttt atc ctt tca atc tat tta ttt att 1180 Tyr Phe Leu Trp Phe Ile Leu Phe Ile Leu Ser Ile Tyr Leu Phe Ile 1228 acc ata caa gaa aga cga ggt tat tgt ttt gac aaa cgt gca tat att Thr Ile Gln Glu Arg Arg Gly Tyr Cys Phe Asp Lys Arg Ala Tyr Ile 30 35 cat gag ctt tat act gag caa gag tta att gat cgg ggg att gaa tat 1276 His Glu Leu Tyr Thr Glu Gln Glu Leu Ile Asp Arg Gly Ile Glu Tyr 1324 gtg gta tcc acc atg ccg tca ggt gtt att aaa cca gat ggc aca ata Val Val Ser Thr Met Pro Ser Gly Val Ile Lys Pro Asp Gly Thr Ile 1372 aaa gaa gta aag cgt tac acg agt gtc gag gag ttt aaa cag atg aac Lys Glu Val Lys Arg Tyr Thr Ser Val Glu Glu Phe Lys Gln Met Asn cca gct tgt tgt aca tta acc acc ttt att gat gaa gga ggc gat ggc 1420 Pro Ala Cys Cys Thr Leu Thr Thr Phe Ile Asp Glu Gly Gly Asp Gly 90 1468 tat cca gat gat gga tat ggt tat gtc aga att gaa tat tta aga Tyr Pro Asp Asp Gly Tyr Gly Tyr Val Arg Ile Glu Tyr Leu Arg 110 cat tat gtt gag aat cta aaa cct tat cat aga gtg att tat ctt gaa 1516 His Tyr Val Glu Asn Leu Lys Pro Tyr His Arg Val Ile Tyr Leu Glu

135

130

125

tat acg ccc tgt gga gag tta agg gaa gag gcg gct ttt tca aaa aat 1564 Tyr Thr Pro Cys Gly Glu Leu Arg Glu Glu Ala Ala Phe Ser Lys Asn 140 145 150

taaqagtgag gtgaagaaat ggcattacca acagcaacaa taatgaggaa tttatcttta 1624 tctaaaaatc aattcactct gaaagggatg gaatgcgtag attccctatt tcaagcatgc 1684 agtaatatgg atcatgggta ctgaggtgga agatggcaga agaaaataaa ggaaagagat 1744 attttttatq qttcatattg tttatccttt caatctattt atttattacc atacaagaaa 1804 qacqaqqtta ttqttttgac aaatgggaat atatccataa cctttatacc gagcaagagt 1864 tgatcgatag aggggttgaa tatgtggtat ccaccatgcc gtcaggtgtt tttgaaccag 1924 atgqcacaac aaccqaaata aaacqttatg ctagtgttga ggagtttaaa cagatgaacc 1984 ctgattgttg taaattaaca agatttatta atgaaggaat agatggctat ccagatgatg 2044 atggatatgg ttatataaga attgaatatt taagacatta tgttgggaat tttaaacctg 2104 atcatagagt gctttatctc gaatatacgc cttgtggaga attaagggaa gaggtttctt 2164 tttaaaaaat aaataatagt gaggtgaaga aatggcatta ccaacagcaa cagaaatcac 2224 aaatqcatat ttatataaaa ataaattaac tcctaaagcg gaggaaagag tagattcaat 2284 acaaattott gaaaaaggag atgaacattt cgaagtaaat tttaattgat caaagtactc 2344 tattgattga aggaaaaaca gtggaattaa tggcaggtat ggcagtttct gcggaaatta 2404 aaacaggtaa acgcagtgta ttagattact tatttagccc attaaaaacc acaaaataat 2464 attaaggaga ataatatgtc gtataataaa tatactgttg ctttgattac gttctcaaca 2524 gggatctgta ttccggcaat atgctacgct ctaaattcgc tgggatacag atcctgtttg 2584 agactatgta gaaaagacta aactttgtgt ggttaactgg gcttcggtaa aattctggaa 2644 acaaatgggc ttaacccqcq tqatcttatc ccqtgagctt tcgcttgatg aaattgccga 2704 aattcgtcag caagtgccag aaatggaaat tgaagtgttc gtgcatgggg cattatgcat 2764 ggcgtattct ggacgttgtt tattatcagg ctatattaat aaacgtgatc caaatcaagg 2824 cacctgtacc aatgcgtgcc gttgggaata cagtgtaacc gaagccaaag aagatgagat 2884 cggcaacatt gtgaatgtgg gtgaagaaat tccagtgaaa aatgtagcac cgacacttgg 2944 cqaaqqcqac accaccaqta aagtattttt attagcagaa agtcga 2990

<sup>&</sup>lt;210> 34

<sup>&</sup>lt;211> 153

<sup>&</sup>lt;212> PRT

<sup>&</sup>lt;213> Pasteurella multocida

<sup>&</sup>lt;400> 34

Met Thr Glu Glu Asn Lys Gly Lys Arg Tyr Phe Leu Trp Phe Ile Leu 1 5 10 15

Phe	Ile	Leu	Ser 20	Ile	Tyr	Leu	Phe	Ile 25	Thr	Ile	Gln	Glu	Arg 30	Arg	Gly	
Tyr	Cys	Phe 35	Asp	Lys	Arg	Ala	Tyr 40	Ile	His	Glu	Leu	Tyr 45	Thr	Glu	Gln	
Glu	Leu 50	Ile	Asp	Arg	Gly	Ile 55	Glu	Tyr	Val	Val	Ser 60	Thr	Met	Pro	Ser	
Gly 65	Val	Ile	Lys	Pro	Asp 70	Gly	Thr	Ile	Lys	Glu 75	Val	Lys	Arg	Tyr	Thr 80	
Ser	Val	Glu	Glu	Phe 85	Lys	Gln	Met	Àsn	Pro 90	Ala	Cys	Cys	Thr	Leu 95	Thr	
Thr	Phe	Ile	Asp 100	Glu	Gly	Gly	Asp	Gly 105	Tyr	Pro	Asp	Asp	Asp 110	Gly	Tyr	
Gly	Tyr	Val 115	Arg	Ile	Glu	Tyr	Leu 120	Arg	His	Tyr	Val	Glu 125	Asn	Leu	Lys	
Pro	Tyr 130	His	Arg	Val	Ile	Tyr 135	Leu	Glu	Tyr	Thr	Pro 140	Cys	Gly	Glu	Leu	
Arg 145	Glu	Glu	Ala	Ala	Phe 150	Ser	Lys	Asn								
<pre> &lt;210&gt; 35 &lt;211&gt; 1683 &lt;212&gt; DNA &lt;213&gt; Pasteurella multocida  &lt;220&gt; &lt;221&gt; CDS &lt;222&gt; (325)(1230)  &lt;220&gt; &lt;223&gt; lgtC</pre>																
	> 35 caaa		ctcat	ggca	a ga	aaaat	taga	a aaa	agago	cgat	caat	tatt	tat t	tgca	aagatt	60
tggg	tatt	at t	cata	aggct	a gg	gtgaa	aagat	ata	atttt	tcc	atga	atatt	caa a	aacga	attcag	120
gcag	aact	gg d	ctago	ttat	c ac	etttt	agat	: aat	tgta	atta	ttaa	aaaga	aag o	ctgta	atgatt	180
gtta	ttct	at o	catta	gtgg	ga ta	ataa	atat	tct	ttat	ttt	ttga	agaga	ata a	aaaa	caattc	240
atat	ttca	at a	igaaa	acag	ja aa	ataa	agat	tat	caaa	aaga	atta	atcc	gtc o	cttat	aaata	300
tgag	rtetg	gta t	tgtg	gagat	g at		_	aat a Asn 1			_	_	_	-		351
				cat His												399
				att Ile 30												447

Glu				aat Asn												495
				gct Ala												543
				att Ile												591
				gat Asp												639
				aac Asn 110												687
				gca Ala												735
tct Ser	gag Glu	cat His 140	aaa Lys	aaa Lys	tcg Ser	att Ile	tca Ser 145	atg Met	tca Ser	gat Asp	aag Lys	gaa Glu 150	tat Tyr	tat Tyr	ttt Phe	783
				atg Met												831
gta Val 170	ttc Phe	tca Ser	aga Arg	gct Ala	tta Leu 175	gac Asp	ctg Leu	tta Leu	gct Ala	atg Met 180	tat Tyr	cct Pro	aat Asn	caa Gln	atg Met 185	879
			gat	caa				aat	atc	ctt		agg	aat	aaa	gtc	927
	IYL	Gln		Gln 190	Asp	Ile	Leu		Ile 195	Leu	Phe					
~	tat	tta	Asp gat		aga	ttt	aat	Asn ttc	195 atg	cca	aat	Arg	Asn	Lys 200 gaa	Val aga	975
Cys ata	tat Tyr aan	tta Leu caa	Asp gat Asp 205 tac	190 tgc	aga Arg aaa	ttt Phe gga	aat Asn aaa	Asn ttc Phe 210 ntg	195 atg Met agc	cca Pro aac	aat Asn tta	Arg caa Gln cat	Asn ctt Leu 215 tct	Lys 200 gaa Glu tta	Val aga Arg gaa	975 1023
Cys ata Ile aaa	tat Tyr aan Xaa	tta Leu caa Gln 220	Asp gat Asp 205 tac Tyr	190 tgc Cys	aga Arg aaa Lys	ttt Phe gga Gly	aat Asn aaa Lys 225 att	Asn ttc Phe 210 ntg Xaa tca	atg Met agc Ser	cca Pro aac Asn	aat Asn tta Leu	caa Gln cat His 230	Asn ctt Leu 215 tct Ser cca	Lys 200 gaa Glu tta Leu gaa	aga Arg gaa Glu	
Cys ata Ile aaa Lys	tat Tyr aan Xaa aca Thr 235	tta Leu caa Gln 220 acg Thr	gat Asp 205 tac Tyr atg Met	tgc Cys cat His	aga Arg aaa Lys gtc Val	ttt Phe gga Gly gtt Val 240	aat Asn aaa Lys 225 att Ile	Asn ttc Phe 210 ntg Xaa tca Ser	atg Met agc Ser cat His	cca Pro aac Asn tat Tyr	aat Asn tta Leu tgt Cys 245	caa Gln cat His 230 ggt Gly	Asn ctt Leu 215 tct Ser cca Pro	Lys 200 gaa Glu tta Leu gaa Glu	aga Arg gaa Glu aaa Lys	1023
Cys ata Ile aaa Lys gcg Ala 250 ata	tat Tyr aan Xaa aca Thr 235 tgg Trp	tta Leu caa Gln 220 acg Thr cat His	gat Asp 205 tac Tyr atg Met gcg Ala	tgc Cys cat His cct Pro	aga Arg aaa Lys gtc Val tgt Cys 255 tcg	ttt Phe gga Gly gtt Val 240 aaa Lys	aat Asn aaa Lys 225 att Ile cat His	Asn ttc Phe 210 ntg Xaa tca Ser ttt Phe ncg	195 atg Met agc Ser cat His aat Asn	cca Pro aac Asn tat Tyr gta Val 260 aaa	aat Asn tta Leu tgt Cys 245 tat Tyr	caa Gln cat His 230 ggt Gly ttc Phe cgc	Asn ctt Leu 215 tct Ser cca Pro tat Tyr	Lys 200 gaa Glu tta Leu gaa Glu cag Gln tta	aga Arg gaa Glu aaa Lys aaa Lys 265	1023

285 290 295

aaa tat caa gtc tat taactattga atttttgcaa atgagataag agtatagtgc 1270 Lys Tyr Gln Val Tyr

tgatttette aaagegaaaa ggaggaaata gettgtteta atttattaca ataatggttg 1330
tatteatett gattttgaag gaaagaggt gttttttgta taaaageatt ttegteacet 1390
aaatttaeta ateeteeaaa tteteeteet egnagaattt ettteggaee ggtagggeag 1450
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<210> 36

<211> 302

<212> PRT

<213> Pasteurella multocida

<400> 36

Met Asn Ile Leu Phe Val Ser Asp Asp Val Tyr Ala Lys His Leu Val

Val Ala Ile Lys Ser Ile Ile Asn His Asn Glu Lys Gly Ile Ser Phe 20 25 30

Tyr Ile Phe Asp Leu Gly Ile Lys Asp Glu Asn Lys Arg Asn Ile Asn 35 40 45

Asp Ile Val Ser Ser Tyr Gly Ser Glu Val Asn Phe Ile Ala Val Asn 50 55 60

Glu Lys Glu Phe Glu Ser Phe Pro Val Gln Ile Ser Tyr Ile Ser Leu 65 70 75 80

Ala Thr Tyr Ala Arg Leu Lys Ala Ala Glu Tyr Leu Pro Asp Asn Leu 85 90 95

Asn Lys Ile Ile Tyr Leu Asp Val Asp Val Leu Val Phe Asn Ser Leu
100 105 110

Glu Met Leu Trp Asn Val Asp Val Asn Asn Phe Leu Thr Ala Ala Cys 115 120 125

Tyr Asp Ser Phe Ile Glu Asn Glu Lys Ser Glu His Lys Lys Ser Ile 130 135 140

Ser Met Ser Asp Lys Glu Tyr Tyr Phe Asn Ala Gly Val Met Leu Phe 145 150 155 160

Asn Leu Asp Glu Trp Arg Lys Met Asp Val Phe Ser Arg Ala Leu Asp 165 170 175

Leu Leu Ala Met Tyr Pro Asn Gln Met Ile Tyr Gln Asp Gln Asp Ile 180 185

Leu	Asn	Ile 195	Leu	Phe	Arg	Asn	Lys 200	Val	Cys	Tyr	Leu	Asp 205	Cys	Arg	Phe	
Asn	Phe 210	Met	Pro	Asn	Gln	Leu 215	Glu	Arg	Ile	Xaa	Gln 220	Tyr	His	Lys	Gly	
Lys 225	Xaa	Ser	Asn	Leu	His 230	Ser	Leu	Glu	Lys	Thr 235	Thr	Met	Pro	Val	Val 240	
Ile	Ser	His	Tyr	Cys 245	Gly	Pro	Glu	Lys	Ala 250	Trp	His	Ala	Asp	Cys 255	Lys	
His	Phe	Asn	Val 260	Tyr	Phe	Tyr	Gln	Lys 265	Ile	Leu	Ala	Xaa	Xaa 270	Ser	Arg	
Gly	Xaa	Asp 275	Lys	Glu	Arg	Val	Leu 280	Ser	Ile	Lys	Thr	Tyr 285	Leu	Lys	Ala	
Leu	Ile 290	Arg	Arg	Ile	Arg	Tyr 295	Lys	Phe	Lys	Tyr	Gln 300	Val	Tyr			
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<pre>&lt;213&gt; Pasteurella multocida  &lt;220&gt; &lt;221&gt; CDS &lt;222&gt; (2)(499)</pre>																
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c tt		at aa	_		-				r Va					Ly As	ac caa sn Gln 15	49
~ ~			_		_	_	_		aaa Lys		_					97
									tta Leu							145
														+ ~+		
	_	-	-				_		atg Met	_	_				_	193
Trp	Asp 50 aaa	Ala	Ala	Leu caa	Ala	Lys 55 gaa	Asp	Lys atc	_	Asp	Ala 60 aac	Trp	Leu gat	Ser ggt	Ser	193 241
Trp tct Ser 65	Asp 50 aaa Lys atg	Ala gca Ala ggg	Ala aat Asn gca	Leu caa Gln ttg	Ala att Ile 70 gaa	Lys 55 gaa Glu gcc	Asp gtg Val acg	Lys atc Ile aaa	Met	Asp gct Ala 75 cat	Ala 60 aac Asn	Trp aac Asn	Leu gat Asp	Ser ggt Gly tta	ser atg Met 80	

ggt gaa att gca ggt acg gtg tta aat gac ggt gtg aac caa ggt aaa 385 Gly Glu Ile Ala Gly Thr Val Leu Asn Asp Gly Val Asn Gln Gly Lys 115 120 125
gcc gtt gtt caa tta agt aat cat ctt gca aaa gga aaa cct gcc act 433 Ala Val Val Gln Leu Ser Asn Asn Leu Ala Lys Gly Lys Pro Ala Thr 130 135 140
gaa ggc aca aaa tgg cag tta aaa cga tcg tgt cct acg tat ccc tta Glu Gly Thr Lys Trp Gln Leu Lys Arg Ser Cys Pro Thr Tyr Pro Leu 145 150 155 160
tgt tgg tgt gga tgc gga taacttaaac gagttcctaa aataataaac 529 Cys Trp Cys Gly Cys Gly 165
tataacaaaa caagamgttg taattctcgg ggaggtatac cctcccctt tttatgtgag 589
gttggatatg acaactcaaa ttccaaatca agacagtgaa atactgctca caatgaccaa 649
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<210> 38

<211> 166

<212> PRT

<213> Pasteurella multocida

<400> 38

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Gly His Pro Asp Ala Glu Ala Arg Thr Lys Phe Val Ile Lys Glu Leu 20 25 30

Xaa Asn Lys Gly Ile Gln Asp Glu Gln Leu Phe Ile Asp Thr Gly Met
35 40 45

Trp Asp Ala Ala Leu Ala Lys Asp Lys Met Asp Ala Trp Leu Ser Ser 50 55 60

Ser Lys Ala Asn Gln Ile Glu Val Ile Ile Ala Asn Asn Asp Gly Met 65 70 75 80

Ala Met Gly Ala Leu Glu Ala Thr Lys Ala His Gly Lys Lys Leu Pro 85 90 95

Ile Phe Xaa Val Xaa Ala Leu Pro Glu Val Leu Gln Leu Ile Lys Lys
100 105 110

Gly Glu Ile Ala Gly Thr Val Leu Asn Asp Gly Val Asn Gln Gly Lys 115 120 125

Ala Val Val Gln Leu Ser Asn Asn Leu Ala Lys Gly Lys Pro Ala Thr 130 135 140

Glu Gly Thr Lys Trp Gln Leu Lys Arg Ser Cys Pro Thr Tyr Pro Leu 145 150 155 160

Cys Trp Cys Gly Cys Gly 165

<210> 39

<211> 2628

<212> DNA

<213> Pasteurella multocida

<220>

<221> CDS

<222> (326)..(766)

<220>

<223> mioC

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<sup>&</sup>lt;211> 147

<sup>&</sup>lt;212> PRT

<sup>&</sup>lt;213> Pasteurella multocida

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Ala Leu Lys Ser Ile Ala Ala Val Gly Arg Asp Ala Lys Leu Met Leu 50 55 60

Gly Arg Thr Pro Lys Ser Ile Ala Ala Ile Arg Pro Met Lys Asp Gly 65 70 75 80

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Lys Gln Val His Ser Ser Asn Phe Met Arg Pro Ser Pro Arg Val Leu
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<213> Pasteurella multocida

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Asp Val Ile Ala Gln Ile Thr Ala Glu Val Ala Glu Gly Glu Asp Ile 50 55 60

Ser Glu Gly Lys Ile Val Asp Ile Phe Thr Ala Leu Glu Ser Gln Ile 65 70 75 80

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Val Asp Thr Val Arg Ala Leu Asp Ile Cys Thr Gly Val Leu Pro Arg
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Tyr Ser Val Gly Glu Thr Gly Met Ile Gly Ser Pro Lys Arg Arg Glu 165 170 175

Ile Gly His Gly Arg Leu Ala Lys Arg Gly Val Ala Ala Val Met Pro 180 185 190

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Glu Ser Asn Gly Ser Ser Ser Met Ala Ser Val Cys Gly Ala Ser Leu 210 215 220

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Tyr Lys Gly Lys Val Thr Arg Leu Ala Asp Phe Gly Ala Phe Val Ser
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Gly Glu Lys Ile Ala Arg Glu Trp Ala Asp Val Asp Asp Ile Asp Val
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	tta Leu															337
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	gct Ala 130															433
	acc Thr			_	_		_			_	_	_	_	_		481
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80

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Arg Tyr Asp Ile Ser Asn Leu Tyr Ile Arg Asp Leu Arg Lys Glu Asp 50 55

Phe Glu Glu Trp Ile Arg Ile Arg Leu Thr Glu Val Ser Asp Ala Ser 65 70 75 80

Val Arg Arg Glu Leu Val Thr Ile Ser Ser Val Leu Thr Thr Ala Ile 85 90 95

Asn Lys Trp Gly Tyr Ile Ser Arg His Pro Met Thr Gly Ile Glu Lys 100 105 110

Pro Lys Asn Ser Ala Glu Arg Lys Glu Arg Tyr Ser Glu Gln Asp Ile 115 120 125

Lys Thr Ile Leu Glu Thr Ala Arg Tyr Cys Glu Asp Lys Leu Pro Ile 130 135 140

Thr Leu Lys Gln Arg Val Ala Ile Ala Met Leu Phe Ala Ile Glu Thr 145 150 155 160

Ala Met Arg Ala Gly Glu Ile Ala Ser Ile Lys Trp Asp Asn Val Phe 165 170 175

Leu Glu Lys Arg Ile Val His Leu Pro Thr Thr Lys Asn Gly His Ser 180 185 190

Arg Asp Val Pro Leu Ser Gln Arg Ala Val Ala Leu Ile Leu Lys Met 195 200 205

Lys Glu Val Glu Asn Gly Asp Leu Val Phe Gln Thr Thr Pro Glu Ser 210 215 220

Leu Ser Thr Thr Phe Arg Val Leu Lys Lys Glu Cys Gly Leu Glu His 225 230 235 240

Leu His Phe His Asp Thr Arg Arg Glu Ala Leu Thr Arg Leu Ser Lys 245 250 255

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Asn Gln Ala Ile Arg Thr Ile Gln Ser Leu Ser Thr Ala Val Ile Gly
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Ile Val Cys Thr Ala Asn Asp Ala Asp Asn Glu Thr Phe Pro Leu Asn
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Asn Cys Lys Val Ile Val Val Arg Val Gln Glu Ser Ala Gln Glu Asp
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577

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Lys Gln Gly Thr Leu Ser Arg Ala Leu Asp Gly Ile Ser Asp Val Val 65 70 75 80

Asn Cys Lys Val Ile Val Val Arg Val Gln Glu Ser Ala Gln Glu Asp 85 90 95

Glu Glu Thr Lys Ala Ser Glu Met Asn Thr Ala Ile Ile Gly Thr Ile 100 105 110

Thr Glu Glu Gly Gln Tyr Thr Gly Leu Lys Ala Leu Leu Ile Ala Lys 115 120 125

Asn Lys Phe Gly Ile Lys Pro Arg Ile Leu Cys Val Pro Lys Phe Asp 130 135 140

Thr Lys Glu Val Ala Thr Glu Leu Ala Ser Ile Ala Ala Lys Leu Asn 145 150 155 160

Ala Phe Ala Tyr Ile Ser Cys Gln Gly Cys Lys Thr Lys Glu Gln Ala 165 170 175

Val Gln Tyr Lys Arg Asn Phe Ser Gln Arg Glu Val Met Leu Ile Met 180 185 190

Gly Asp Phe Leu Ser Phe Asn Val Asn Thr Ser Lys Val Glu Ile Asp 195 200 205

Tyr Ala Val Thr Arg Ala Ala Ala Met Arg Ala Tyr Leu Asp Lys Glu 210 215 220

Gln Gly Trp His Thr Ser Ile Ser Asn Lys Gly Ile Asn Gly Val Ser 225 230 235 240

Gly Val Thr Gln Pro Leu Tyr Phe Asp Ile Asn Asp Ser Ser Thr Asp 245 250 255

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	Val	Lys	Asp	Ile	Ile 325	Glu	Ala	Ile	Asn	Ala 330	Lys	Trp	Arg	Asp	Tyr 335	Thr	
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	gat Asp 65	agc Ser	tct Ser	aat Asn	ata Ile	cct Pro 70	ttg Leu	ttt Phe	agg Arg	agt Ser	aat Asn 75	tgg Trp	gaa Glu	ttg Leu	att Ile	atc Ile 80	240
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gat gat gaa aag tta atg atg gaa tta ttt cct gaa gat aaa gta aga
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Tyr Phe Leu Glu Lys Lys Glu Glu Phe Asn Phe Gln Asp Tyr Ser Phe
Glu Glu Met Tyr Ile Phe Ser Lys Met Glu Pro Val Tyr Val Leu Cys
Asp Ser Ser Asn Ile Pro Leu Phe Arg Ser Asn Trp Glu Leu Ile Ile
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gat aag gaa att ata tta gaa ttc gaa aat gaa ttt aat ata aag ctt
                                                                   96
Asp Lys Glu Ile Ile Leu Glu Phe Glu Asn Glu Phe Asn Ile Lys Leu
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Pro Se	r Leu 35		Ile	Asp	Leu	Ile 40		Ala	His	Asn	Ala 45	Pro	Lys	Ser	
Glu Gl 5	u Asn 0	Cys	Phe	Glu	Tyr 55	Tyr	Asn	Glu	Arg	Asn 60		Pro	Thr	Phe	
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                                                                192
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Ile Tyr Tyr Leu Phe Tyr Lys Arg Gly Val Glu Phe Cys Phe Lys Arg
ata gat gaa gag tat gtc tta tat tcg gtt ttc ttt ttc ttg gta gag
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Ile Asp Glu Glu Tyr Val Leu Tyr Ser Val Phe Phe Leu Val Glu
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gtt gat aat tat ttt tca tgc cca ttt att cat gaa tta ata tgt gat
Val Asp Asn Tyr Phe Ser Cys Pro Phe Ile His Glu Leu Ile Cys Asp
                                                                336
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Leu Lys His Gly Phe Ser Ile Glu Asp Ile Ile Arg Phe Leu Gly Glu
           100
cca aat ttt aaa ggt agt ggc tgg gta aga tat tct tat aat gga aga
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			cgg Arg								3561
			caa Gln								3609
			aca Thr								3657
			ttc Phe								3705
			tgt Cys 345								3753
			aag Lys								3801
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Gly Asp Val Asn Arg Gln Val Val Ser Pro Gln Asp Lys Ala Lys Phe 85 90 95

Gly Gly Asn Glu Phe Met Ala Lys Gln Glu Lys Arg Asn Gln Glu Leu 100 105 110

Ile Gln Gly Ile Ala Lys Leu Tyr Leu Arg Ser Glu Asn Ala Asn Ala 115 120 125

Ser Ser Asp Ala Pro Ile Thr Ile Asp Lys Pro Phe His Tyr Ser Cys 130 135 140

Glu Glu Leu Asp Leu Pro Thr Ala Asn Glu Tyr Ala Arg Arg Lys Pro 145 150 155 160

Ile Val Cys Glu Val Gln Gly Gly Val Asn Arg Lys Phe Trp Leu Pro 165 170 175

Val Ser Glu Ser Leu Val Ser Glu Asp Lys Leu Lys Lys Asp Arg Val 180 185 190

Arg Leu Glu Ser Asp Thr Ser Tyr Ala Ile Lys Glu Lys Gly Ile Val

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859

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<211> 257

<212> PRT

<213> Pasteurella multocida

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20 25 30

Ser Met Ser Ser Glu Thr Ile Thr Ala Lys Glu Thr Leu Tyr Glu Ser 35 40 45

Thr Gln Asn Tyr Ser Ala Leu Ile Ser Leu Tyr Arg Asp Val Leu Lys
50 60

Ala Lys Glu Asp Pro Ser Ile Arg Tyr Lys Leu Ala Lys Thr Tyr Tyr 65 70 75 80

Gln Arg Gly Asp Ser Lys Ser Ser Leu Leu Tyr Leu Thr Pro Leu Leu
85 90 95

Asn Asp Asn Thr Lys Leu Ala Thr Gln Ala Lys Ile Leu Gln Ile Lys 100 105 110

Asn Leu Ile Gln Leu Asn Asn Phe Gln Glu Ala Ile Ser Val Ala Asn 115 120 125

Glu Leu Leu Lys Ser Pro Asn Glu Gly Glu Val Tyr Asn Leu Arg 130 135 140

Gly Ile Ala Tyr Ala Gln Asn Gly Asn Leu Val Asn Ala Arg Asn Asp 145 150 155 160

Ile Asn Lys Ala Arg Glu Phe Phe Ile Asn Asp Asn Val Ala Ile Asn 165 170 175

Asn Leu Ala Met Leu Asn Ile Ile Asn Gly Asp Phe Asn Asn Ala Val 180 185 190

Ser Leu Leu Pro Gln Tyr Leu Asn Gly Val Lys Asn Ser Arg Leu 195 200 205

Ile His Asn Leu Val Phe Ala Leu Val Lys Asn Gly Asp Leu Asp Tyr 210 215 220

Ala Lys Asp Ile Ile Val Lys Glu Arg Leu Asn Thr Ser Pro Asp Asp

Arg

<210> 62

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<213> Pasteurella multocida

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13

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1 10 15

aaa gat gac acc agt ttt gtg act gaa gga aat aac ttt atc aca gca 96 Lys Asp Asp Thr Ser Phe Val Thr Glu Gly Asn Asn Phe Ile Thr Ala

aaa gac aac tta gaa atc acg gca aaa aat gtt caa att gat caa gcg 144 Lys Asp Asn Leu Glu Ile Thr Ala Lys Asn Val Gln Ile Asp Gln Ala 35 40 45

aaa aat att caa tta aac gcg aat atc acg atc aat acc aag tct ggt Lys Asn Ile Gln Leu Asn Ala Asn Ile Thr Ile Asn Thr Lys Ser Gly

ttt gtg aat tac ggt acc tta gca agt gct caa aat tta acg att aat 240 Phe Val Asn Tyr Gly Thr Leu Ala Ser Ala Gln Asn Leu Thr Ile Asn 65 70 75 80

acc gaa caa ggc agc att tat aac ata ggc ggt atc ttg ggg gcg ggt 288
Thr Glu Gln Gly Ser Ile Tyr Asn Ile Gly Gly Ile Leu Gly Ala Gly
85
90
95

ctt att aat caa ggt aag agt cta ctc cat tct gaa ggc gcc atg aac
Leu Ile Asn Gln Gly Lys Ser Leu Leu His Ser Glu Gly Ala Met Asn
115 120 125

ctc aca gcg gat cgc acg gtg tac aat tta ggg aat att ttt gct aaa 432 Leu Thr Ala Asp Arg Thr Val Tyr Asn Leu Gly Asn Ile Phe Ala Lys 130 135 140

ggt gac gcg acg atc aat gca aac gcg tta att aat gat gtt act ctc 480 Gly Asp Ala Thr Ile Asn Ala Asn Ala Leu Ile Asn Asp Val Thr Leu 145 150 155 160

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528
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Thr Gly Arq Leu Glu Tyr Gln Asp Leu Lys Lys Asp Tyr Thr Arg Tyr
                165
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                                                        175
tat cgt atc aat gaa acg gca aaa cat ggt tgg cat aat aac ttc tat
                                                                  576
Tyr Arg Ile Asn Glu Thr Ala Lys His Gly Trp His Asn Asn Phe Tyr
gaa tta aac gtc gac aga gtt tct tgatttgtgc atcaattttg taaccaccgg
Glu Leu Asn Val Asp Arg Val Ser
        195
ttaataaaac accagcaatt tcaacgccat tcatggcaga taatgccgct gcgacgatca 690
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ttcatgacca aagegageeg etttgttttt atetgaatee aettgataae egaacagttt 1770
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<sup>&</sup>lt;210> 63

<sup>&</sup>lt;211> 200

<sup>&</sup>lt;212> PRT

<sup>&</sup>lt;213> Pasteurella multocida

<sup>&</sup>lt;400> 63

Val Asn Thr Gly Leu Ile His Ser Asn Gly Asn Ala Lys Leu Thr Phe

1 5 10 15

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Lys	Asp	Asn 35	Leu	Glu	Ile	Thr	Ala 40	Lys	Asn	Val	Gln	Ile 45	Asp	Gln	Ala	
Lys	Asn 50	Ile	Gln	Leu	Asn	Ala 55	Asn	Ile	Thr	Ile	Asn 60	Thr	Lys	Ser	Gly	
Phe 65	Val	Asn	Tyr	Gly	Thr 70	Leu	Ala	Ser	Ala	Gln 75	Asn	Leu	Thr	Ile	Asn 80	-
Thr	Glu	Gln	Gly	Ser 85	Ile	Tyr	Asn	Ile	Gly 90	Gly	Ile	Leu	Gly	Ala 95	Gly	
Lys	Ser	Leu	Asn 100	Leu	Ser	Ala	Lys	Arg 105	Gly	Glu	Asn	Gln	Gly 110	Gly	Tyr	
Leu	Ile	Asn 115	Gln	Gly	Lys	Ser	Leu 120	Leu	His	Ser	Glu	Gly 125	Ala	Met	Asn	
Leu	Thr 130	Ala	Asp	Arg	Thr	Val 135	Tyr	Asn	Leu	Gly	Asn 140	Ile	Phe	Ala	Lys	
Gly 145	Asp	Ala	Thr	Ile	Asn 150	Ala	Asn	Ala	Leu	Ile 155	Asn	Asp	Val	Thr	Leu 160	
Thr	Gly	Arg	Leu	Glu 165	Tyr	Gln	Asp	Leu	Lys 170	Lys	Asp	Tyr	Thr	Arg 175	Tyr	
Tyr	Arg	Ile	Asn 180	Glu	Thr	Ala	Lys	His 185	Gly	Trp	His	Asn	Asn 190	Phe	Tyr	
Glu	Leu	Asn 195	Val	Asp	Arg	Val	Ser 200									
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<220 <221 <222	- CI		. (27	78)												
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	)> 64 tcca		aaat	ctca	ıc ac	ccaga	agcaa	a gaa	acgct	caca	tagt	ggaa	atg 9	gttgg	gcagaa	60
catt	acco	aa a	ıtgga	aata	a ac	ctta	aacca	a tag	gcaag	gaga	gaag	gaaa	_	aaa Lys		116
							gaa Glu									164
							gcc Ala									212

1.71 1.4

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:: :::

: Th

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gct ttg att gag ggt gtc gag cac ttt gtg ctg gaa ggt gag gaa
Ala Leu Ile Glu Gly Val Glu His Phe Val Leu Glu Gly Glu Glu Glu
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agc aaa agg gga cat agt
Ser Lys Arg Gly His Ser
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<211> 57
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<213> Pasteurella multocida
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Leu Thr Leu Ala Leu Ile Glu Gly Val Glu His Phe Val Leu Glu Gly
Glu Glu Glu Ser Lys Arg Gly His Ser
<210> 66
<211> 1020
<212> DNA
<213> Pasteurella multocida
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<222> (1)..(597)
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<223> unknown P
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                                                                   96
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Ala Met Arg Ala Tyr Leu Asp Lys Glu Gln Gly Trp His Thr Ser Ile
tca aat aaa ggc att aat ggc gtg agc ggt gtc aca caa cca ctc tat
                                                                   144
Ser Asn Lys Gly Ile Asn Gly Val Ser Gly Val Thr Gln Pro Leu Tyr
                             40
                                                                   192
ttt gac att aac gac agc tcg act gat gtg aac tat ctc aat gaa caa
Phe Asp Ile Asn Asp Ser Ser Thr Asp Val Asn Tyr Leu Asn Glu Gln
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cgc Arg	acg Thr	act Thr	gca Ala	gaa Glu 85	gat Asp	cca Pro	tta Leu	ttc Phe	aag Lys 90	ttt Phe	gaa Glu	gtg Val	tac Tyr	acc Thr 95	cgc Arg	288
														tgg Trp		336
gtg Val	gat Asp	aaa Lys 115	gat Asp	att Ile	tct Ser	gtc Val	acg Thr 120	cta Leu	gtg Val	aaa Lys	gat Asp	att Ile 125	att Ile	gaa Glu	gca Ala	384
atc Ile	aat Asn 130	gcg Ala	aag Lys	tgg Trp	cgt Arg	gat Asp 135	tac Tyr	acc Thr	aca Thr	aaa Lys	ggc Gly 140	tac Tyr	tta Leu	att Ile	ggc Gly	432
ggt Gly 145	aaa Lys	gcg Ala	tgg Trp	ctt Leu	aat Asn 150	aaa Lys	gag Glu	ctt Leu	aac Asn	agt Ser 155	gca Ala	acg Thr	aat Asn	tta Leu	aaa Lys 160	480
gat Asp	gcg Ala	aag Lys	ttg Leu	ttg Leu 165	atc Ile	tct Ser	tat Tyr	gat Asp	tat Tyr 170	cac His	cca Pro	gta Val	cca Pro	ccg Pro 175	ctc Leu	528
gaa Glu	cag Gln	cta Leu	ggc Gly 180	ttt Phe	aat Asn	cag Gln	tac Tyr	att Ile 185	tct Ser	gat Asp	gaa Glu	tac Tyr	ctt Leu 190	gtt Val	gat Asp	576
	tca Ser						taag	33331	tag a	aaaat	tggc	tt ta	acca	cgcaa	a	627
acti	caaat	tg a	atgaa	attta	aa to	catc	gacg	g ta	acaa	atat	ctc	ggcg	aag	tcac	ggaagt	687
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ggt	cgaat	ta a	attaa	aaaa	at to	egge	gggt	c aa	tcaa	cggc	att	ccat	tgc	gttt	tcttgg	867
ctca	atato	cag o	egtga	atga	ca ca	agaa	gaag	t ca	catc	tgtt	gag	cttg	tga	tgca	aggtcg	927
att	tacto	gaa a	attga	acago	eg ga	aaac	agca	a ag	tggg	cgat	gac	actg	aac	aaac	attcaa	987
agt	gcctt	ta a	acgta	atta	ca a	aatc	attg	t tg	a							1020

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<211> 199

<212> PRT

<213> Pasteurella multocida

<400> 67

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Ser Asn Lys Gly Ile Asn Gly Val Ser Gly Val Thr Gln Pro Leu Tyr 35 40 45

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Phe Asp Ile Asn Asp Ser Ser Thr Asp Val Asn Tyr Leu Asn Glu Gln 50 55 60
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Gly Ile Thr Cys Cys Val Asn His Asn Gly Phe Arg Phe Trp Gly Leu 65 70 75 80

Arg Thr Thr Ala Glu Asp Pro Leu Phe Lys Phe Glu Val Tyr Thr Arg
85 90 95

Thr Ala Gln Ile Leu Lys Asp Thr Ile Ala Gly Ala Phe Asp Trp Ala
100 105 110

Val Asp Lys Asp Ile Ser Val Thr Leu Val Lys Asp Ile Ile Glu Ala 115 120 125

Ile Asn Ala Lys Trp Arg Asp Tyr Thr Thr Lys Gly Tyr Leu Ile Gly 130 135 140

Gly Lys Ala Trp Leu Asn Lys Glu Leu Asn Ser Ala Thr Asn Leu Lys 145 150 155 160

Asp Ala Lys Leu Leu Ile Ser Tyr Asp Tyr His Pro Val Pro Pro Leu 165 170 175

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175 180 185

ttg tgg gga g Leu Trp Gly G	gga cgt gaa Gly Arg Glu 190	Gly Tyr	gaa acg Glu Thr 195	tta tta Leu Leu	aat acc Asn Thr 200	aat tta Asn Leu	1647
aaa cag gag G Lys Gln Glu <i>I</i> 205	cga gag caa Arg Glu Gln	att gga Ile Gly 210	cgt ttc Arg Phe	atg caa Met Gln	atg gtg Met Val 215	gtt gag Val Glu	1695
cat aaa tat a His Lys Tyr I 220	aaa atc ggt Lys Ile Gly	ttt aac Phe Asn 225	ggg act Gly Thr	ttg ctg Leu Leu 230	att gaa Ile Glu	cca aag Pro Lys	1743.
cca caa gag c Pro Gln Glu F 235	cca acg aaa Pro Thr Lys 240	cat caa His Gln	tat gac Tyr Asp	tat gat Tyr Asp 245	gtg gcg Val Ala	acc gtt Thr Val 250	
tat ggc ttt t Tyr Gly Phe I	tta aag cag Leu Lys Gln 255	ttt ggt Phe Gly	tta gaa Leu Glu 260	aaa gaa Lys Glu	att aaa Ile Lys	gtg aat Val Asn 265	1839
att gaa gct a Ile Glu Ala A	aat cac gca Asn His Ala 270	Thr Leu	gct gga Ala Gly 275	cac act His Thr	ttc cag Phe Gln 280	cat gaa His Glu	1887
gtc gcc atg g Val Ala Met A 285	gct aca gcg Ala Thr Ala	tta gat Leu Asp 290	att ttt Ile Phe	ggt tct Gly Ser	att gat Ile Asp 295	gca aat Ala Asr	1935
cgt ggt gat o Arg Gly Asp I 300	cca caa tta Pro Gln Leu	ggt tgg Gly Trp 305	gat acc Asp Thr	gat caa Asp Gln 310	ttc cct Phe Pro	aat ago Asn Ser	1983
gta gaa gaa a Val Glu Glu <i>I</i> 315							<b>-</b>
ttt aca acc o	ggt ggt ttt Gly Gly Phe 335	aat ttt Asn Phe	gat gct Asp Ala 340	aaa atc Lys Ile	cgt cgg Arg Arg	cag agt Gln Ser 345	2079
acg gat cct t Thr Asp Pro 3		Phe His					
ctt gcc tta t Leu Ala Leu S 365.	tca cta aaa Ser Leu Lys	tgt gcg Cys Ala 370	gcg aaa Ala Lys	atg ctt Met Leu	gaa gag Glu Glu 375	caa gct Gln Ala	2175 i
tta caa aaa g Leu Gln Lys V 380							
ggt caa ctt g Gly Gln Leu V 395							3
cta aca aaa c Leu Thr Lys V		aacgttc c	ggcttacg	gc cagaca	atcta ga	cgattgaa	a 2326

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<213> Pasteurella multocida

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Ile Leu Gly Lys Thr Met Ala Glu His Leu Arg Leu Ala Val Cys Tyr 35 40 45

Trp His Thr Phe Cys Trp Thr Gly Asn Asp Met Phe Gly Val Gly Ser
50 55 60

Phe Asp Arg Cys Trp Gln Lys Ala Ser Asp Ser Leu Ala Gly Ala Lys 65 70 75 80

Gln Lys Ala Asp Ile Ala Phe Glu Phe Phe Ser Lys Leu Gly Ile Pro 85 90 95

Tyr Tyr Cys Phe His Asp Val Asp Val Ala Pro Glu Gly His Ser Phe 100 105 110

Lys Glu Tyr Leu Ser Asn Phe Asn Thr Met Ile Asp Val Leu Ala Gln 115 120 125

Lys Gln Glu Glu Thr Gly Val Lys Leu Leu Trp Gly Thr Ala Asn Cys 130 135 140

Phe Thr His Pro Arg Tyr Met Ser Gly Ala Ala Thr Asn Pro Asn Pro 145 150 155 160

Glu Ile Phe Ala Trp Ala Ala Ala Gln Val Phe Thr Ala Met Gly Ala 165 170 175

Thr Gln Arg Leu Gly Gly Glu Asn Tyr Val Leu Trp Gly Gly Arg Glu 180 185 190

Gly Tyr Glu Thr Leu Leu Asn Thr Asn Leu Lys Gln Glu Arg Glu Gln
195 200 205

Ile Gly Arg Phe Met Gln Met Val Val Glu His Lys Tyr Lys Ile Gly 210 220

Phe Asn Gly Thr Leu Leu Ile Glu Pro Lys Pro Gln Glu Pro Thr Lys 225 230 235 240

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His Gln Tyr Asp Tyr Asp Val Ala Thr Val Tyr Gly Phe Leu Lys Gln
                245
Phe Gly Leu Glu Lys Glu Ile Lys Val Asn Ile Glu Ala Asn His Ala
                                265
Thr Leu Ala Gly His Thr Phe Gln His Glu Val Ala Met Ala Thr Ala
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Leu Asp Ile Phe Gly Ser Ile Asp Ala Asn Arg Gly Asp Pro Gln Leu
                        295
Gly Trp Asp Thr Asp Gln Phe Pro Asn Ser Val Glu Glu Asn Thr Leu
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305
Val Ile Tyr Glu Ile Leu Lys Ala Gly Gly Phe Thr Thr Gly Gly Phe
                                     330
Asn Phe Asp Ala Lys Ile Arg Arg Gln Ser Thr Asp Pro Tyr Asp Leu
Phe His Gly His Ile Gly Ala Ile Asp Val Leu Ala Leu Ser Leu Lys
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Cys Ala Ala Lys Met Leu Glu Glu Gln Ala Leu Gln Lys Val Val Asn
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atg ttt aag cga ttt cgt gca ttc aca tac cgt ccc gcc agt tat ctt
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393

Met Phe Lys Arg Phe Arg Ala Phe Thr Tyr Arg Pro Ala Ser Tyr Leu

ggc ggg atg ttg gtg att gtt ttt ctg agc gct ttt tat gcg ttc gcc

Gly	Gly	Met	Leu 20	Val.	Ile	Val	Phe	Leu 25	Ser	Ala	Phe	Tyr	Ala 30	Phe	Ala	
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ttg Leu	agt Ser 50	gat Asp	cag Gln	tat Tyr	tta Leu	caa Gln 55	cac His	gtg Val	atc Ile	atc Ile	ttt Phe 60	agc Ser	ttt Phe	tgg Trp	caa Gln	489
gcc Ala 65	Phe	ctg Leu	tcg Ser	gcg Ala	gta Val 70	ctt Leu	gcg Ala	gtc Val	ctc Leu	ttt Phe 75	ggt Gly	ggc Gly	att Ile	gta Val	gca Ala 80	537
cga Arg	gcc Ala	ttt Phe	ttt Phe	tat Tyr 85	caa Gln	ccg Pro	ttt Phe	gtg Val	ggc Gly 90	aag Lys	aaa Lys	ctg Leu	atc Ile	ctc Leu 95	aaa Lys	585
tta Leu	ttt Phe	tca Ser	ctg Leu 100	act Thr	ttt Phe	gtg Val	tta Leu	cct Pro 105	gcc Ala	tta Leu	gtg Val	gcg Ala	att Ile 110	ttt Phe	ggt Gly	633
tta Leu	tta Leu	ggc Gly 115	gtg Val	tat Tyr	ggc Gly	gct Ala	tct Ser 120	ggc Gly	tgg Trp	tta Leu	gcg Ala	atg Met 125	tta Leu	agc Ser	cag Gln	681
ttt Phe	ttc Phe 130	gct Ala	tgg Trp	gat Asp	tgg Trp	act Thr 135	cct Pro	aat Asn	att Ile	tac Tyr	ggc Gly 140	tta Leu	aca Thr	ggt Gly	att Ile	729
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caa Gln	ctc Leu	aat Asn	tta Leu 180	cgt Arg	ggt Gly	tgg Trp	cat His	ttt Phe 185	ata Ile	cgt Arg	ctg Leu	att Ile	gag Glu 190	tgg Trp	ccc Pro	873
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tgt Cys	ttt Phe 210	acc Thr	agt Ser	ttt Phe	gcg Ala	att Ile 215	gtg Val	ctc Leu	act Thr	tta Leu	ggt Gly 220	ggc Gly	gga Gly	ccg Pro	aaa Lys	969
tat Tyr 225	acc Thr	acg Thr	ttg Leu	gaa Glu	gtg Val 230	gct Ala	atc Ile	tat Tyr	caa Gln	gcg Ala 235	att Ile	tta Leu	ttt Phe	gag Glu	ttt Phe 240	1017
gat Asp	gta Val	ccg Pro	aaa Lys	gcc Ala 245	ggc Gly	tta Leu	ttt Phe	gcg Ala	tta Leu 250	Leu	caa Gln	ttt Phe	gtt Val	ttt Phe 255	tgt Cys	1065

aca Thr	tta Leu	cac His 275	agt Ser	caa Gln	cct Pro	act Thr	tgg Trp 280	ttt Phe	gcg Ala	ccc Pro	caa Gln	tcg Ser 285	tat Tyr	tgg Trp	gtt Val	1161.
												gta Val				1209
tta Leu 305	ecg Pro	cta Leu	ctc Leu	aat Asn	acg Thr 310	cta Leu	gtt Val	tct Ser	gct Ala	ttg Leu 315	ctt Leu	tcg Ser	tct Ser	cag Gln	ttt Phe 320	1257.
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ctc Leu	acc Thr	atc Ile	gcc Ala 340	ccc Pro	act Thr	tct Ser	gca Ala	ttg Leu 345	ctc Leu	gct Ala	tta Leu	gta Val	ctg Leu 350	tct Ser	ttt Phe	1353
gcc Ala	tta Leu	tta Leu 355	ttg Leu	ctt Leu	gcc Ala	aga Arg	gaa Glu 360	tta Leu	cat His	tgg Trp	cga Arg	cat His 365	tat Tyr	cgc Arg	agc Ser	1401
tta Leu	tcc Ser 370	cat His	gtg Val	att Ile	tta Leu	aat Asn 375	atc Ile	ggt Gly	gcg Ala	acc Thr	att Ile 380	tta Leu	gcc Ala	att Ile	cca Pro	1449
acg Thr 385	tta Leu	gtg Val	tta Leu	gct Ala	att Ile 390	ggt Gly	tta Leu	ttc Phe	att Ile	tta Leu 395	tta Leu	cgt Arg	gag Glu	atc Ile	gat Asp 400	1497
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gct Ala	gct Ala	atg Met	cct Pro 420	ttt Phe	gtg Val	ttg Leu	cgt Arg	att Ile 425	ttg Leu	gct Ala	tta Leu	ccg Pro	atg Met 430	cat His	aac Asn	1593
aat Asn	atg Met	att Ile 435	tat Tyr	tat Tyr	gaa Glu	aaa Lys	tta Leu 440	tgc Cys	caa Gln	tca Ser	ctt Leu	aac Asn 445	ctg Leu	cgt Arg	ggt Gly	1641
tgg Trp	caa Gln 450	cgt Arg	ttt Phe	cga Arg	ttg Leu	att Ile 455	gaa Glu	tgg Trp	cac His	aag Lys	ctt Leu 460	cgt Arg	gcg Ala	cca Pro	atg Met	1689
aaa Lys 465	tac Tyr	gcc Ala	ttt Phe	gca Ala	ctg Leu 470	gct Ala	tgt Cys	gcg Ala	tta Leu	tca Ser 475	tta Leu	ggc	gat Asp	ttc Phe	acc Thr 480	1737
gca Ala	atc Ile	gcg Ala	tta Leu	ttt Phe 485	ggt Gly	cag Gln	gct Ala	gac Asp	ttc Phe 490	aca Thr	tcg Ser	tta Leu	ccg Pro	cat His 495	ttg Leu	1785
ttg Leu	tat Tyr	caa Gln	caa Gln 500	ttg Leu	gly ggg	cat His	tat Tyr	cgt Arg 505	agt Ser	cag Gln	gaa Glu	gcg Ala	gca Ala 510	gta Val	aca Thr	1833
gcg Ala	ttt Phe	att Ile	tta Leu	ttg Leu	gtt Val	ttt Phe	tgt Cys	ttg Leu	agt Ser	gtt Val	ttt Phe	atg Met	att Ile	att Ile	gaa Glu	1881

515 520 525

cga cat cag gaa ccg cgt gat gat taatttaaac ggtgttcagt tttcctataa 1935 Arg His Gln Glu Pro Arg Asp Asp 530 535

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Leu Ser Asp Gln Tyr Leu Gln His Val Ile Ile Phe Ser Phe Trp Gln 50 60

Ala Phe Leu Ser Ala Val Leu Ala Val Leu Phe Gly Gly Ile Val Ala 65 70 75 80

Arg Ala Phe Phe Tyr Gln Pro Phe Val Gly Lys Lys Leu Ile Leu Lys 85 90 95

Leu Phe Ser Leu Thr Phe Val Leu Pro Ala Leu Val Ala Ile Phe Gly
100 105 110

Leu Leu Gly Val Tyr Gly Ala Ser Gly Trp Leu Ala Met Leu Ser Gln 115 120 125

Phe Phe Ala Trp Asp Trp Thr Pro Asn Ile Tyr Gly Leu Thr Gly Ile 130 135 140

Leu Leu Ala His Leu Phe Phe Asn Val Pro Leu Ala Cys Arg Leu Phe 145 150 155 160

Leu Gln Gly Leu Gln Ala Ile Pro Val Gln Gln Arg Gln Leu Ala Ala 165 170 175

Gln Leu Asn Leu Arg Gly Trp His Phe Ile Arg Leu Ile Glu Trp Pro 180 185 190

Tyr Leu Arg Gln Gln Leu Leu Pro Ala Phe Thr Leu Ile Phe Met Leu 195 200 205

Cys Phe Thr Ser Phe Ala Ile Val Leu Thr Leu Gly Gly Pro Lys 210 215 220

Tyr Thr Thr Leu Glu Val Ala Ile Tyr Gln Ala Ile Leu Phe Glu Phe 225 230 235 240

Asp Val Pro Lys Ala Gly Leu Phe Ala Leu Leu Gln Phe Val Phe Cys 245 250 255

Phe Leu Leu Phe Thr Leu Ser Ser Phe Phe Ser Pro Ala Pro Ala Thr 260 265 270

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Ala Leu Leu Leu Ala Arg Glu Leu His Trp Arg His Tyr Arg Ser
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Leu Ser His Val Ile Leu Asn Ile Gly Ala Thr Ile Leu Ala Ile Pro
Thr Leu Val Leu Ala Ile Gly Leu Phe Ile Leu Leu Arg Glu Ile Asp
Phe Ser Pro Tyr His Leu Phe Gly Val Val Val Cys Cys Asn Ala Leu
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Trp Gln Arg Phe Arg Leu Ile Glu Trp His Lys Leu Arg Ala Pro Met
Lys Tyr Ala Phe Ala Leu Ala Cys Ala Leu Ser Leu Gly Asp Phe Thr
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Ala Ile Ala Leu Phe Gly Gln Ala Asp Phe Thr Ser Leu Pro His Leu
Leu Tyr Gln Gln Leu Gly His Tyr Arg Ser Gln Glu Ala Ala Val Thr
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tta Leu	ggt Gly	ttt Phe 55	ctc Leu	act Thr	gly aaa	tta Leu	atc Ile 60	gct Ala	tta Leu	gtt Val	att Ile	tca Ser 65	tat Tyr	ctt Leu	tgg Trp	1747
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gca Ala	aca Thr	tta Leu	ctt Leu	gat Asp 105	aag Lys	act Thr	gga Gly	att Ile	gct Ala 110	aga Arg	gat Asp	ctc Leu	tac Tyr	aac Asn 115	gca Ala	1891
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tac Tyr	ttc Phe 230	aaa Lys	cct Pro	agc Ser	tat Tyr	ggc Gly 235	cct Pro	gca Ala	atg Met	cct Pro	agc Ser 240	tca Ser	gaa Glu	aat Asn	cat His	2275
aca Thr 245	tta Leu	acg Thr	aaa Lys	gaa Glu	gat Asp 250	att Ile	aaa Lys	aaa Lys	att Ile	att Ile 255	cat His	gat Asp	att Ile	gca Ala	att Ile 260	2323
cca Pro	gta Val	gct Ala	atc Ile	gcc Ala 265	aca Thr	tgg Trp	att Ile	tta Leu	gga Gly 270	agt Ser	att Ile	tat Tyr	ggc	999 Gly 275	ata Ile	2371
gca Ala	tca Ser	atc Ile	act Thr 280	gaa Glu	tct Ser	gcc Ala	tgt Cys	gtt Val 285	ggt Gly	gta Val	gtt Val	Gly 999	gta Val 290	ata Ile	tta Leu	2419
gca Ala	gca Ala	ttc Phe	tat Tyr	cga Arg	aaa Lys	gaa Glu	tta Leu	aat Asn	ttc Phe	aaa Lys	ata Ile	gta Val	caa Gln	gaa Glu	tca Ser	2467

295 300 305

cta aaa cat aca atc aat act gtt ggt atg at	a atc tgg gtc ggc att 2515
Leu Lys His Thr Ile Asn Thr Val Gly Met Il 310 315	e Ile Trp Val Gly Ile 320
ggc gca aca atg att ata ggt att tat aat ct Gly Ala Thr Met Ile Ile Gly Ile Tyr Asn Le	a atg ggt ggg gac cga 2563 u Met Gly Gly Asp Arg
325 330 33	
ttt ata gct aac tta ttc gct agc tta gat gc Phe Ile Ala Asn Leu Phe Ala Ser Leu Asp Al	la Ser Pro Ile Tyr Thr
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atc att att atg atg gtt att tta tta ata ct Ile Ile Ile Met Met Val Ile Leu Leu Ile Le	
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tgg att ggt gtt gcc atg ttg act ttc ctc aa Trp Ile Gly Val Ala Met Leu Thr Phe Leu Ly	ag aca agt aaa gcg aca 2707 ys Thr Ser Lys Ala Thr
375 380	385
atc aat ttg tgt ttt gac ata gtc agg tac ag Ile Asn Leu Cys Phe Asp Ile Val Arg Tyr Se	gt att tgg cgt ggt ccc 2755 er Ile Trp Arg Gly Pro
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405 410 43	15 420
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<213> Pasteurella multocida

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Gly Ile Gly Thr Leu Ile Ile Phe Leu Met Met Ile Ser Leu Leu Phe

Ile Gly Met Pro Leu Gly Phe Leu Thr Gly Leu Ile Ala Leu Val Ile

Ser 65	Tyr	Leu	Trp	Phe	Asp 70	Thr	Thr	Ala	Ile	Met 75	Gln	Met	Ile	Ala	Ser 80
Arg	Val	Thr	Asp	Phe 85	Thr	Ser	Ser	Tyr	Thr 90	Phe	Val	Ala	Val	Pro 95	Met
Phe	Val	Leu	Met 100	Ala	Thr	Leu	Leu	Asp 105	Lys	Thr	Gly	Ile	Ala 110	Arg	Asp
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Met	Leu	Arg	Leu	Gly 165	Tyr	Asn	Lys	Asn	Leu 170	Ala	Ile	Gly	Thr	Val 175	Val
Ala	Gly	Gly	Ala 180	Leu	Gly	Thr	Met	Val 185	Pro	Pro	Ser	Ile	Val 190	Leu	Ile
Ile	Tyr	Gly 195	Met	Thr	Ala	Asn	Val 200	Ser	Ile	Gly	Glu	Leu 205	Phe	Leu	Ala
Ala	Ile 210	Pro	Ala	Ser	Leu	Leu 215	Leu	Ser	Thr	Phe	Tyr 220	Ile	Leu	Tyr	Ile
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Ser	Glu	Asn	His	Thr 245	Leu	Thr	Lys	Glu	Asp 250	Ile	Lys	Lys	Ile	Ile 255	His
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Trp	Val	Gly	Ile	Gly 325	Ala	Thr	Met	Ile	Ile 330	Gly	Ile	Tyr	Asn	Leu 335	Met
Gly	Gly	Asp	Arg 340	Phe	Ile	Ala	Asn	Leu 345	Phe	Ala	Ser	Leu	Asp 350	Ala	Ser
Pro	Ile	Tyr 355	Thr	Ile	Ile	Ile	Met 360	Met	Val	Ile	Leu	Leu 365	Ile	Leu	Gly
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                                                Met Val Leu Pro
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Ile Ile Ser Thr Pro Lys Leu Trp Gln Tyr Ile Pro Ser Ser Lys Leu
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Met Ala Ser Arg Val Val Gly Arg Thr Arg Ser Val Pro Ser Lys Ala
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                                  45
                                                                    666
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Ile Ile Ser Ala Pro Ala Ala Ala Asn Ser Ser Met Ser Cys Lys Asn
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         55
ggg cta ata cga acg gga ctg tca ggt aaa tcg cgt tta acg ata cca
                                                                    714
Gly Leu Ile Arg Thr Gly Leu Ser Gly Lys Ser Arg Leu Thr Ile Pro
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ata atc ggt aca ttg acg acg tta cgc gtg gct ttt aaa ttt tcg atc
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Pro Ser Lys Ala Ile Ile Ser Ala Pro Ala Ala Ala Asn Ser Ser Met
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Ser Cys Lys Asn Gly Leu Ile Arg Thr Gly Leu Ser Gly Lys Ser Arg 65 70 75 80

Leu Thr Ile Pro Ile Ile Gly Thr Leu Thr Thr Leu Arg Val Ala Phe
85 90 95

Lys Phe Ser Ile Pro Ser Ile Arg Asn Pro Ala Ala Pro Pro Ile Thr 100 105 110

Asp Ala Cys Ala Met Ala Ala Thr Ile Ser Gly Glu Ser Ile Gly Pro 115 120 125

Leu Ser Thr Gly Trp Gln Asp Ala Ile Lys Pro Tyr Leu Ile Cys Ser 130 135 140

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<sup>&</sup>lt;211> 158

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<sup>&</sup>lt;213> Pasteurella multocida

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<sup>&</sup>lt;211> 2787

<sup>&</sup>lt;212> DNA

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<211> 279

<212> PRT

<213> Pasteurella multocida

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Val Lys Glu Lys Ser Asn Gly Lys Ile Asp Val Ala Ile Phe Pro Ser 50 55 60

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Ala Leu Asp Phe Thr Leu Gly Glu Ser Ala Arg Phe Gln Ile Tyr Phe 85 90 95

Pro Glu Ala Glu Val Phe Ala Leu Pro Tyr Met Ile Pro Asn Phe Glu 100 105 110

Thr Ser Lys Lys Ala Leu Leu Asp Thr Lys Phe Gly Gln Gly Leu Leu 115 120 125

Lys Lys Ile Asp Lys Glu Leu Asn Val Gln Val Leu Ser Val Ala Tyr 130 135 140

Asn Gly Thr Arg Gln Thr Thr Ser Asn Arg Ala Ile Asn Ser Ile Glu 145 150 155 160

Asp Met Lys Gly Leu Lys Leu Arg Val Pro Asn Ala Ala Thr Asn Leu 165 170 175

Ala Tyr Ala Lys Tyr Val Gly Ala Ala Pro Thr Pro Met Ala Phe Ser 180 \$185

Glu Val Tyr Leu Ala Leu Gln Thr Asn Ser Val Asp Gly Gln Glu Asn

195 200 205

Pro Leu Pro Thr Ile Gln Ala Gln Lys Phe Tyr Glu Val Gln Lys Tyr 210 215 220

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<212> DNA

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gta gca caa gca cgt caa tcg tta gaa aa Val Ala Gln Ala Arg Gln Ser Leu Glu As 50 55	
caa gcg gga tta caa gtc gca aat atc gt Gln Ala Gly Leu Gln Val Ala Asn Ile Va 65 70	g aaa acc acg gtg ttt gtg 1141 al Lys Thr Thr Val Phe Val 75
aaa gat tta aat gac ttt gca gcg gtc aa Lys Asp Leu Asn Asp Phe Ala Ala Val As 80 85	
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Ile Pro Val Asn Pro Lys Thr Gly Glu Val Pro Ala Asp Ile Val Ala 35 40 45

Gln Ala Arg Gln Ser Leu Glu Asn Val Lys Ala Ile Val Glu Gln Ala 50 - 55 60

Gly Leu Gln Val Ala Asn Ile Val Lys Thr Thr Val Phe Val Lys Asp 65 70 75 80

Leu Asn Asp Phe Ala Ala Val Asn Ala Glu Tyr Glu Arg Phe Phe Lys 85 90 95

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Arg Leu Pro Lys Asp Val Gly Ile Glu Ile Glu Ala Ile Ala Val Lys 115 120 125

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<212> DNA

<213> Pasteurella multocida

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<400> 80

<sup>&</sup>lt;211> 129

<sup>&</sup>lt;212> PRT

<sup>&</sup>lt;213> Pasteurella multocida

165

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170

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										cgt Arg						1146
_			_	_		-				agt Ser						1194
	Asp									acg Thr 255						1242
										gat Asp						1290
										aaa Lys						1338
										agc Ser						1386
		_		_		_	_	_		gag Glu			_			1434
	_		_			_	_	_		agc Ser 335					_	1482
										gat Asp						1530
										caa Gln						1578
										aca Thr						1626
										gaa Glu						1674
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425 430 435

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gtg gga cgt tat cac gcg tagtcgtgct atcccttcaa atatttaacc 1914 Val Gly Arg Tyr His Ala 470

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<sup>&</sup>lt;211> 474

<sup>&</sup>lt;212> PRT

<sup>&</sup>lt;213> Pasteurella multocida

<sup>&</sup>lt;400> 81

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Tyr Asp Ser Ser Lys Met Ala Asp Leu Leu Asn Ser Thr His Gly Leu 20 25 30

Glu Leu Thr Glu Ile Pro Glu Glu Ala Asp Val Leu Leu Leu Asn Thr

35 40 45

Cys Ser Ile Arg Glu Lys Ala Gln Glu Lys Val Phe His Gln Leu Gly Arg Trp Lys Glu Leu Lys Lys His Lys Pro Gly Leu Val Ile Gly Val Gly Gly Cys Val Ala Ser Gln Glu Gly Glu His Ile Arg Thr Arg Ala Pro Tyr Val Asp Ile Ile Phe Gly Pro Gln Thr Leu His Arg Leu Pro Glu Met Ile Asn Gln Ile Arg Gly Gly Lys Ser Ser Val Val Asp Val Ser Phe Pro Glu Ile Glu Lys Phe Asp Arg Leu Pro Glu Pro Arg Ala Glu Gly Pro Thr Ala Phe Val Ser Ile Met Glu Gly Cys Asn Lys Tyr 150 Cys Ser Phe Cys Val Val Pro Tyr Thr Arg Gly Glu Glu Val Ser Arg Pro Val Asp Asp Val Leu Phe Glu Ile Ala Gln Leu Ala Glu Gln Gly 185 Val Arg Glu Val Asn Leu Leu Gly Gln Asn Val Asn Ala Tyr Arg Gly 200 Ala Thr His Asp Asp Gly Ile Cys Thr Phe Ala Glu Leu Leu Arg Leu Val Ala Ile Asp Gly Ile Asp Arg Leu Arg Phe Thr Thr Ser His Pro Ile Glu Phe Thr Asp Asp Ile Ile Asp Val Tyr Arg Asp Thr Pro 245 250 Glu Leu Val Ser Phe Leu His Leu Pro Val Gln Ser Gly Ser Asp Arg Val Leu Ser Met Met Lys Arg Asn His Thr Ala Leu Glu Tyr Lys Ser 275 Ile Ile Arg Lys Leu Arg Ala Val Arg Pro Glu Ile Gln Ile Ser Ser 295 300 Asp Phe Ile Val Gly Phe Pro Gly Glu Thr Ala Glu Asp Phe Glu Gln Thr Met Asn Leu Ile Ala Gln Val Asn Phe Asp Met Ser Phe Ser Phe 330 Ile Tyr Ser Ala Arg Pro Gly Thr Pro Ala Ala Asp Met Pro Asp Asp Val Thr Glu Glu Lys Lys Gln Arg Leu Tyr Val Leu Gln Gln Arg 360

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Gln Arg Val Leu Val Glu Gly Pro Ser Lys Lys Asp Leu Met Glu Leu
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Asp Met Ile Gly Lys Phe Val Asp Ile Lys Ile Thr Asp Val Phe Thr
Asn Ser Leu Arg Gly Glu Val Val Arg Thr Glu Glu Gln Met Gly Leu
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tgatgcaggt cagttagcaa actatctcac tttagatgcg acagacccaa tttcaaaaga 300
aacggacttc aaaaaatgtg cggtcaaagt ggaaaaagcg taacacgtta aatttaatga 360
ggaacgaccg cactttgctt tcagtaaagt gcggttggaa agtcga atg aaa aaa
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                                                   Met Lys Lys
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                                                                   463
Thr Val Val Asn Pro Glu Arg Arg Phe Phe Lys Glu Ala Thr Arg
                                                                   511
act gca ggc ggg ttg gca ggg gtg act ttg ctc ctt ggt ttg caa caa
Thr Ala Gly Gly Leu Ala Gly Val Thr Leu Leu Gly Leu Gln Gln
aag cag agt ctt gcg cgc gaa ggc gtg gcg tta cgc cca cct ttt gcc
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Lys Gln Ser Leu Ala Arg Glu Gly Val Ala Leu Arg Pro Pro Phe Ala
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tca cc Ser Pro 8	Met	gaa Glu	gca Ala	ggt Gly	aca Thr 90	ccg Pro	tat Tyr	ttc Phe	att Ile	gcg Ala 95	cgc Arg	gat Asp	aag Lys	ccc Pro	703
tgt gaa Cys Gli 100	a atg ı Met	tgt Cys	gtg Val	gat Asp 105	att Ile	cct Pro	tgt Cys	gca Ala	aaa Lys 110	gcc Ala	tgc Cys	cca Pro	acc Thr	ggt Gly 115	751
gca tto Ala Le	g gat ı Asp	aat Asn	caa Gln 120	gca Ala	aca Thr	gaa Glu	atc Ile	gat Asp 125	gat Asp	gcg Ala	cgt Arg	atg Met	999 Gly 130	tta Leu	799
gct gte Ala Va															847
tgt ga Cys As															895
tta gte Leu Va 16	L Met														943
cca aca Pro Th															991
gct tge Ala Cy	_		_	-		_									1039
gcg aaa Ala Ly															1087
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<sup>&</sup>lt;211> 250

<sup>&</sup>lt;212> PRT

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Cys Gly Gln Cys Val Gln Ala Cys Pro His Glu Met Leu His Leu Ala 65 70 75 80

Ser Leu Ile Ser Pro Met Glu Ala Gly Thr Pro Tyr Phe Ile Ala Arg 85 90 95

Asp Lys Pro Cys Glu Met Cys Val Asp Ile Pro Cys Ala Lys Ala Cys
100 105 110

Pro Thr Gly Ala Leu Asp Asn Gln Ala Thr Glu Ile Asp Asp Ala Arg 115 120 125

Met Gly Leu Ala Val Leu Leu Asp His Glu Thr Cys Leu Asn Trp Gln 130 135 140

Gly Leu Arg Cys Asp Val Cys Tyr Arg Val Cys Pro Leu Ile Asn Lys 145 150 155 160

Ala Ile Thr Leu Val Met His Arg Asn Glu Arg Thr Gly Lys His Ala 165 170 175

Val Phe Ile Pro Thr Val His Ser Glu Ala Cys Thr Gly Cys Gly Lys 180 185 190

Cys Glu Glu Ala Cys Val Leu Glu Glu Ala Ala Ile Lys Val Leu Pro 195 200 205

Met Ala Leu Ala Lys Gly Met Leu Gly Lys His Tyr Arg Leu Gly Trp 210 215 220

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Ser Leu Pro Thr Arg Leu Pro Glu Ser Leu 245 250

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His Phe Asp Thr Leu Asn Asn Asn Ala Val Arg Phe Leu Ser Gly Gly 35 40 45												
Ser Val Phe Ile Leu Ala Cys Phe Phe Tyr Tyr Arg Ala Glu Leu Thr 50 55 60												
Ser Ser Gly Ala Gly Val Gln Ser Val Ala Met Leu Pro Ser Ser 65 70 75 80												
Leu Gly Phe Leu Ile Leu Lys Thr Val Pro Ser Phe Ser Tyr Val Thr 85 90 95												
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55

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gcc Ala	gca Ala	tta Leu	aag Lys	caa Gln 85	gtg Val	cct Pro	tca Ser	tgc Cys	gag Glu 90	ttt Phe	tat Tyr	ccg Pro	tta Leu	gag Glu 95	gca Ala	288
											tta Leu					336
caa Gln	aac Asn	cag Gln 115	ata Ile	aaa Lys	cgc Arg	gtc Val	gtc Val 120	tgt Cys	ctt Leu	agc Ser	aca Thr	gat Asp 125	aaa Lys	gcg Ala	gtg Val	384
tac Tyr	cca Pro 130	att Ile	aat Asn	gcg Ala	atg Met	ggc Gly 135	att Ile	tct Ser	aaa Lys	gca Ala	atg Met 140	atg Met	gaa Glu	aaa Lys	gtc Val	432
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aaa Lys 225	gcc Ala	ccc Pro	gca Ala	gca Ala	acc Thr 230	att Ile	ggt Gly	acc Thr	ctt Leu	gcc Ala 235	aaa Lys	gca Ala	att Ile	acc Thr	gaa Glu 240	720
tta Leu	tta Leu	tct Ser	gtc Val	cca Pro 245	aat Asn	cac His	cct Pro	att Ile	tcc Ser 250	att Ile	ata Ile	ggt Gly	acg Thr	cgt Arg 255	cat His	768
											gaa Glu					816
											gcc Ala					864
											cca Pro 300					912
											ttg Leu					960

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Gly Asn Ala Val Leu Lys Arg Phe Leu Glu Thr Asp Ile Arg Glu Ile 20 25 30

Arg Val Phe Ser Arg Asp Glu Lys Lys Gln Asp Asp Met Arg Lys Lys 35 40 45

Tyr Asn Asp Ala Lys Leu Lys Phe Tyr Ile Gly Asp Val Arg Asp Tyr
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Asp Ser Ile Leu Asn Ala Ser Arg Gly Val Asp Tyr Ile Tyr His Ala 65 70 75 80

Ala Ala Leu Lys Gln Val Pro Ser Cys Glu Phe Tyr Pro Leu Glu Ala 85 90 95

Val Lys Thr Asn Ile Leu Gly Thr Ala Asn Val Leu Glu Ala Ala Ile 100 105 110

Gln Asn Gln Ile Lys Arg Val Val Cys Leu Ser Thr Asp Lys Ala Val 115 120 125

Tyr Pro Ile Asn Ala Met Gly Ile Ser Lys Ala Met Met Glu Lys Val 130 135 140

Ile Ile Ala Lys Ser Arg Asn Leu Glu Gly Thr Pro Thr Thr Ile Cys 145 150 155 160

Cys Thr Arg Tyr Gly Asn Val Met Ala Ser Arg Gly Ser Val Ile Pro 165 170 175

Leu Phe Val Asp Gln Ile Arg Gln Gly Lys Pro Phe Thr Ile Thr Asp 180 185 190

Pro Glu Met Thr Arg Phe Met Met Thr Leu Glu Asp Ala Val Asp Leu 195 200 205

Val Leu Tyr Ala Phe Lys Asn Gly Gln Asn Gly Asp Val Phe Val Gln

Lys Ala Pro Ala Ala Thr Ile Gly Thr Leu Ala Lys Ala Ile Thr Glu 225 230 235 240 Leu Leu Ser Val Pro Asn His Pro Ile Ser Ile Ile Gly Thr Arg His 245 250 Gly Glu Lys Ala Phe Glu Ala Leu Leu Ser Arg Glu Glu Met Val His Ala Ile Asn Glu Gly Asn Tyr Tyr Arg Ile Pro Ala Asp Gln Arg Ser Leu Asn Tyr Ser Lys Tyr Val Glu Lys Gly Glu Pro Lys Ile Thr Glu Val Thr Asp Tyr Asn Ser His Asn Thr Glu Arg Leu Thr Val Lys Glu 310 315 Met Lys Gln Leu Leu Lys Leu Glu Phe Ile Gln Lys Met Ile Glu Gly Glu Tyr Ile Ser Pro Glu Val 340 <210> 102 <211> 4931 <212> DNA <213> Pasteurella multocida <220> <223> fhaB2 <220> <221> CDS <222> (1)..(4929) <400> 102 atg aac aaa aat cgt tac aaa ctc att ttt agt caa gtc aaa ggt tgt Met Asn Lys Asn Arg Tyr Lys Leu Ile Phe Ser Gln Val Lys Gly Cys ctc gtt cct gtg gca gaa tgt att aac tca gct att agc aat ggt tca Leu Val Pro Val Ala Glu Cys Ile Asn Ser Ala Ile Ser Asn Gly Ser 20 25 tct gat tca aca tcc aca tca gaa caa gtt gaa gag gaa cct ttc ctt Ser Asp Ser Thr Ser Thr Ser Glu Gln Val Glu Glu Pro Phe Leu 35 192 cta gaa caa tat tca ctt tcc tcc gtg tct tta tta gta aaa agc acg Leu Glu Gln Tyr Ser Leu Ser Ser Val Ser Leu Leu Val Lys Ser Thr 50 240 ttc aat cct gtt tcg tat gca atg caa ttg act tgg aaa cag ctt tct Phe Asn Pro Val Ser Tyr Ala Met Gln Leu Thr Trp Lys Gln Leu Ser 65 att tta ttt tta act qtq att tct qtt cct gtt ttg gct gag gga aaa 288 Ile Leu Phe Leu Thr Val Ile Ser Val Pro Val Leu Ala Glu Gly Lys 85 ggg gat gaa aga aat caa tta aca gtg att gat aat agc gat cat att Gly Asp Glu Arg Asn Gln Leu Thr Val Ile Asp Asn Ser Asp His Ile 100 105 110

aa Ly	a tta s Lei	gat Asp 115	gca Ala	tct Ser	aat Asn	ctt Leu	gct Ala 120	ggt Gly	aat Asn	gat Asp	aaa Lys	aca Thr 125	aaa Lys	atc Ile	tat Tyr	384
ca Gl	a gca n Ala 130	a Glu	aat Asn	aaa Lys	gtt Val	ctg Leu 135	gtt Val	att Ile	gat Asp	att Ile	gct Ala 140	aaa Lys	cca Pro	aat Asn	gly aaa	432
аа Ly 14	a ggg s Gly	g att 7 Ile	tca Ser	gat Asp	aac Asn 150	cgt Arg	ttt Phe	gaa Glu	aaa Lys	ttt Phe 155	aat Asn	att Ile	cca Pro	aat Asn	agc Ser 160	480
gc	g gto a Va.	ttt Phe	aat Asn	aat Asn 165	aat Asn	glà aaa	act Thr	gaa Glu	gcg Ala 170	cag Gln	gca Ala	aga Arg	tca Ser	aca Thr 175	tta Leu	528
at Il	t ggt .e Gly	tac Tyr	att Ile 180	ccg Pro	caa Gln	aat Asn	caa Gln	aat Asn 185	tta Leu	agg Arg	gga Gly	Gly ggg	aaa Lys 190	gaa Glu	gct Ala	576
ga As	it gti sp Val	ata I Ile 195	tta Leu	aat Asn	caa Gln	gtg Val	aca Thr 200	ggt Gly	cct Pro	caa Gln	gaa Glu	agt Ser 205	aaa Lys	att Ile	gtt Val	624
gg Gl	jc gcg y Ala	a Leu	gaa Glu	gta Val	tta Leu	ggt Gly 215	aaa Lys	aaa Lys	gct Ala	gat Asp	atc Ile 220	gtc Val	att Ile	gca Ala	aac Asn	672
ca G1 22	ia aat .n Asi !5	ggt Gly	att Ile	acc Thr	tta Leu 230	aat Asn	ggt Gly	gta Val	aga Arg	aca Thr 235	ata Ile	aat Asn	tca Ser	gat Asp	cgt Arg 240	720
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ga As	it gga sp Gly	a tta 7 Leu 275	aag Lys	tat Tyr	tta Leu	gat Asp	att Ile 280	att Ile	gct Ala	aaa Lys	aaa Lys	att Ile 285	gaa Glu	caa Gln	aag Lys	864
Ca Gl	a tca n Se: 290	: Ile	aca Thr	tca Ser	Gly aaa	gat Asp 295	aat Asn	tca Ser	gaa Glu	gca Ala	aaa Lys 300	aca Thr	gat Asp	gtc Val	act Thr	912
ct Le 30	t ati eu Ile )5	gcg Ala	ggt Gly	tcc Ser	agt Ser 310	gaa Glu	tat Tyr	gat Asp	tta Leu	agc Ser 315	aaa Lys	cat His	gag Glu	ctg Leu	aaa Lys 320	960
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	et ag er Se:															1056
	it aaa sp Lys															1104

355 360 365

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gtg Val 465	gca Ala	aca Thr	gaa Glu	act Thr	cta Leu 470	act Thr	aat Asn	gct Ala	gly ggg	cgt Arg 475	att Ile	tat Tyr	ggt Gly	cga Arg	gag Glu 480	1440
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ctt Leu 545	agt Ser	gca Ala	cag Gln	ttt Phe	aag Lys 550	cct Pro	ggt Gly	ttt Phe	gtg Val	aat Asn 555	aag Lys	gga Gly	ctc Leu	att Ile	gaa Glu 560	1680
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gcc Ala	caa Gln	aat Asn 595	att Ile	gaa Glu	att Ile	gat Asp	aaa Lys 600	aat Asn	caa Gln	gat Asp	att Ile	caa Gln 605	ttg Leu	ggt Gly	gct Ala	1824

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ctg Leu 625	gca Ala	act Thr	ggt Gly	aaa Lys	aca Thr 630	ctg Leu	aca Thr	att Ile	aat Asn	acc Thr 635	gaa Glu	agt Ser	ggc Gly	agt Ser	att Ile 640	1920
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act Thr 705	cat His	aat Asn	ttg Leu	att Ile	aat Asn 710	gat Asp	gtg Val	cgt Arg	tta Leu	tct Ser 715	ggc Gly	aat Asn	gtg Val	agt Ser	tat Tyr 720	2160
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aat Asn	gca Ala	tta Leu	gca Ala 820	agc Ser	gtg Val	ttt Phe	aag Lys	aat Asn 825	cca Pro	gcg Ala	aaa Lys	atc Ile	acg Thr 830	atg Met	tac Tyr	2496
tat Tyr	caa Gln	cca Pro 835	ctt Leu	act Thr	cgt Arg	tat Tyr	att Ile 840	tgg Trp	aca Thr	cca Pro	tta Leu	tcg Ser 845	ggt Gly	aat Asn	gca Ala	2544
tcg Ser	cgt Arg	gaa Glu	ttt Phe	aac Asn	aat Asn	tta Leu	gag Glu	tct Ser	ttc Phe	ctc Leu	gat Asp	gcc Ala	ttg Leu	ttt Phe	ggc Gly	2592

850 855 860

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gct tat cag ctt Ala Tyr Gln Leu	cta tct cat Leu Ser His 885	att cag cat Ile Gln His 890	tca cca atg Ser Pro Met	tac caa aa Tyr Gln Ly 895	aa 2688 78
gcg atg gca caa Ala Met Ala Gln 900	Val Phe Gly	gca gag tgg Ala Glu Trp 905	cat agt aaa His Ser Lys	tcc tat ga Ser Tyr As 910	at 2736 sp
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And the time to th

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Gln Asn Gly Ile Thr Leu Asn Gly Val Arg Thr Ile Asn Ser Asp Arg

225					230					235					240
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Gln	Ser 290	Ile	Thr	Ser	Gly	Asp 295	Asn	Ser	Glu	Ala	Lys 300	Thr	Asp	Val	Thr
Leu 305	Ile	Ala	Gly	Ser	Ser 310	Glu	Tyr	Asp	Leu	Ser 315	Lys	His	Glu	Leu	Lys 320
Lys	Thr	Ser	Gly	Glu 325	Asn	Val	Ser	Asn	Asp 330	Val	Ile	Ala	Ile	Thr 335	Gly
Ser	Ser	Thr	Gly 340	Ala	Met	His	Gly	Lys 345	Asn	Ile	Lys	Leu	Ile 350	Val	Thr
Asp	Lys	Gly 355	Ala	Gly	Val	Lys	His 360	Asp	Gly	Ile	Ile	Leu 365	Ser	Glu	Asn
Asp	Ile 370	Gln	Ile	Glu	Met	Asn 375	Glu	Gly	Asp	Leu	Glu 380	Leu	Gly	Asn	Thr
Ile 385	Gln	Gln	Thr	Val	Val 390	Lys	Lys	Asp	Arg	Asn 395	Ile	Arg	Ala	Lys	Lys 400
Lys	Ile	Glu	Val	Lys 405	Asn	Ala	Asn	Arg	Val 410	Phe	Val	Gly	Ser	Gln 415	Thr
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Ala	Leu 450	Ser	Ile	Glu	Gln	Asn 455	Ala	Lys	Leu	Val	Ala 460	Lys	Lys	Ile	Asp
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Val	Lys	Leu	Asp	Thr 485	Asn	Asn	Leu	Ile	Asn 490	Asp	Lys	Glu	Ile	Tyr 495	Ala
Glu	Arg	Lys	Leu 500	Ser	Ile	Leu	Thr	Lys 505	Gly	Lys	Asp	Leu	Glu 510	Ile	Ile
Gln	Asp	Arg 515	Tyr	Leu	Ser	Pro	Leu 520	Met	Arg	Val	Lys	Ser 525	Ser	Val	Arg
	Leu 530	Gly	Ser	Pro	Phe	Phe 535	Ser	Ile	Ser	Pro	Ser 540	Met	Leu	Ala	Ser
Leu 545	Ser	Ala	Gln	Phe	Lys 550	Pro	Gly	Phe	Val	Asn 555	Lys	Gly	Leu	Ile	Glu 560

Ser Ala Gly Ser Ala Glu Leu Thr Phe Lys Glu Lys Thr Ser Phe Leu 565 570 575

Thr Glu Gly Asn Asn Phe Ile Arg Ala Lys Asp Ala Leu Thr Ile Asn 580 585 590

Ala Gln Asn Ile Glu Ile Asp Lys Asn Gln Asp Ile Gln Leu Gly Ala 595 600 605

Asn Ile Thr Leu Asn Val Glu Glu Asn Phe Val Asn Arg Ala Gly Thr 610 615 620

Leu Ala Thr Gly Lys Thr Leu Thr Ile Asn Thr Glu Ser Gly Ser Ile 625 630 635 640

Tyr Asn Leu Gly Gly Thr Leu Gly Ala Gly Lys Ser Leu Lys Leu Thr 645 650 655

Ala Lys Ser Thr Glu Glu Gly Met Gly Asn Ile Val Asn Gln Glu Asn 660 665 670

Gly Leu Phe His Thr Leu Gly Asn Met Met Leu Glu Ala Glu Arg Ser 675 680 685

Val Tyr Asn Ile Gly Asp Ile Tyr Ala Ser Lys Leu Thr Val His 690 695 700

Thr His Asn Leu Ile Asn Asp Val Arg Leu Ser Gly Asn Val Ser Tyr 705 710 715 720

Lys Pro Ile Gly Ser Ser Arg Asp Tyr Asp Ile Ser Arg Val Ala Val 725 730 735

His Gly Trp His Asn Asn Val Tyr Lys Leu Asn Leu Asn Leu Gln Glu
740 745 750

Gln Asp Lys Thr Asp Ile Lys Val Val Lys Met Gly Ala Ile Arg Ser 755 760 765

Asp Gly Asp Phe Asp Phe Lys Gly Ile Lys Ala Thr Ser Ser Glu Ser 770 780

Lys Pro Gln Leu Ile Asn His Gly Leu Ile Asn Val Lys Gly Thr Phe 785 790 795 800

Asn Ala Glu Ala Asp Gln Val Val Asn Gln Met Lys Ala Phe Asn Gln 805 810 815

Asn Ala Leu Ala Ser Val Phe Lys Asn Pro Ala Lys Ile Thr Met Tyr 820 825 830

Tyr Gln Pro Leu Thr Arg Tyr Ile Trp Thr Pro Leu Ser Gly Asn Ala 835 840 845

Ser Arg Glu Phe Asn Asn Leu Glu Ser Phe Leu Asp Ala Leu Phe Gly 850 855 860

Ser Thr Thr Ile Leu Lys Ser Ser Phe Tyr Ser Thr Glu Asn Phe Ser 865 870 875 880

Ala Tyr Gln Leu Leu Ser His Ile Gln His Ser Pro Met Tyr Gln Lys 885 890 895

- Ala Met Ala Gln Val Phe Gly Ala Glu Trp His Ser Lys Ser Tyr Asp 900 905 910
- Glu Met Arg Asn Lys Trp Lys Ser Phe Lys Glu Asn Pro Thr Asp Phe 915 920 925
- Ile Tyr Tyr Pro Ser Glu Lys Ala Lys Ile Leu Ala Gly Lys Leu Glu 930 935 940
- Gly Lys Leu Thr Thr Leu Gln Asn Gly Glu Tyr Ala Glu Arg Gly Lys 945 950 955 960
- Phe Asp Glu Ser Ile Gln Ile Gly Lys His Gln Leu Ser Leu Pro Ser 965 970 975
- Val Glu Leu Lys Ala Glu Phe Ser Asp Lys Glu Arg Leu Glu Glu Asp 980 985 990
- Gly Val Asp Leu Ser Ser Ile Ala Glu Leu Leu Glu Met Pro Asn Leu 995 1000 1005
- Phe Ile Asp Asn Ser Ile Gln Leu Glu Lys Lys Leu Ser Pro Ile 1010 1020
- Glu Asp Leu Asp Glu Glu Pro Arg Lys Asn Leu Asp Ile Glu Glu Ser 1025 1030 1035 1040
- His Ser Asn Ser Ser Asp Asp Val Leu Ser Met Asn Asp Asp Glu Ser 1045 1050 1055
- Asp Thr Asp Asp Ser Lys Trp Ser Met Gly Asn Asp Glu Lys Glu Met 1060 1065 1070
- Pro Asp Asp Lys Leu Gly Ile Ser Arg Asp Asp Arg Gly Asn Lys Pro 1075 1080 1085
- Pro Arg Thr Asp Pro Thr Val Asp Tyr Leu Asn Pro Asp Glu Phe Phe 1090 1095 1100
- Glu Asn Gly Tyr Leu Leu Asn Glu Leu Leu Gln Glu Leu Gly Glu Glu 1105 1110 1115 1120
- Pro Leu Leu Lys Glu Gly Glu Asp His Phe Lys Arg Ser Thr Asn Leu 1125 1130 1135
- Val Arg Leu Gly Glu Arg Asp Arg Gln Asn Arg Glu Lys 1140 1145 1150
- Glu Gly Tyr Phe Asp Leu Pro Gly Thr Leu Asp Met Lys Leu Gln Glu 1155 1160 1165
- Leu Phe Glu Lys Arg Lys Gln Lys His Glu Ala Glu Gln Lys Ala Arg 1170 1175 1180
- Ile Glu Lys Ala Leu Leu Gln Lys Ser Glu Gln Gln Glu Lys Arg Val 1185 1190 1195 1200
- Glu Glu Arg Lys Gln Glu Glu Lys Arg Gln Ala Gln Asp Lys Ile Ala 1205 1210 1215
- Lys Gln Val Glu Ile Ala Lys Glu Met Gln Arg Val Glu Glu Ile Arg 1220 1225 1230

- Gln Arg Glu Lys Gln Leu Ala Ile Gln Leu Gln Glu Glu Lys Lys 1235 1240 1245
- Gln Gln Glu Glu Lys His Leu Ser Glu Glu Lys Lys Gln Ala Glu Gln 1250 1255 1260
- Lys Gln Lys Ala Glu Glu Lys Val Ala Gln Glu Arg Leu Asp Ile Glu 1265 1270 1275 1280
- Gln Gln Lys Ala Tyr Glu Glu Met Ala Lys Arg Glu Ala Glu Ala Ser 1285 1290 1295
- Lys Asn Val Leu Leu Lys Ala Ile Asp Glu Glu Arg Pro Lys Val Glu 1300 1305 1310
- Thr Asp Pro Leu Phe Arg Thr Lys Leu Lys Tyr Ile Asn Gln Asp Asp 1315 1320 1325
- Tyr Ala Gly Ala Asn Tyr Phe Phe Asn Lys Val Gly Leu Asn Thr Lys 1330 1335 1340
- Gly His Gln Lys Val Asn Val Leu Gly Asp Asn Tyr Phe Asp His Gln 1345 1350 1355 1360
- Val Ile Thr Arg Ser Ile Glu Lys Lys Val Asp Asn His Leu Asn Gln 1365 1370 1375
- Lys Tyr Asn Leu Ser Asp Val Glu Leu Val Lys Gln Leu Met Asp Asn 1380 1385 1390
- Ser Thr Thr Gln Ala Gln Glu Leu Asp Leu Lys Leu Gly Ala Ala Leu 1395 1400 1405
- Thr Lys Glu Gln Gln Ala Asn Leu Thr Gln Asp Ile Val Trp Tyr Val
- Lys Thr Lys Val Lys Gly Lys Asp Val Phe Val Pro Lys Val Tyr Phe 1425 1430 1435 1440
- Ala Ser Glu Thr Leu Val Glu Ala Gln Lys Leu Gln Gly Leu Gly Thr 1445 1450 1455
- Gly Thr Ile Arg Val Gly Glu Ala Lys Ile Lys Ala Lys Asp Val Val 1460 1465 1470
- Asn Thr Gly Thr Leu Ala Gly Arg Lys Leu Asn Val Glu Ala Ser Asn 1475 1480 1485
- Lys Ile Lys Asn Gln Gly Ser Ile Leu Ser Thr Gln Glu Thr Arg Leu 1490 1495 1500
- Val Gly Arg Lys Gly Ile Glu Asn Val Ser Arg Ser Phe Ala Asn Asp 1505 1510 1515 1520
- Glu Leu Gly Val Thr Ala Gln Arg Ser Glu Ile Lys Thr Glu Gly His 1525 1530 1535
- Leu His Leu Glu Thr Asp Lys Asp Ser Thr Ile Asp Val Gln Ala Ser 1540 1545 1550
- Asp Ile Lys Ala Lys Thr Ser Phe Val Lys Thr Gly Asp Val Asn Leu 1555 1560 1565

Lys Asn Thr Tyr Asn Thr Lys His Ala Tyr Arg Glu Lys Phe Ser Pro 1575 Ser Ala Leu Gln Val Ala Glu Leu Asp Val Ala Gly Leu Lys Val Pro Leu Leu Gly Val Ser Val Ser Ile Gln Phe Ile Gln Ser Ile Leu Val Arg Gln Leu Gln Glu Gly Ser Ile Phe Glu Val Gly His Leu His Xaa 1625 Ala Val Asp Arg Arg Cys Glu Pro Ser Gly Glu 1640 <210> 104 <211> 2009 <212> DNA <213> Pasteurella multocida <220> <223> hmbR <220> <221> CDS <222> (1)..(2007) <400> 104 atc cgt ggc gtt gat aaa gat cgt gtc gct gtt att gtt gat gga ata Ile Arg Gly Val Asp Lys Asp Arg Val Ala Val Ile Val Asp Gly Ile 10 ccg cag gct gaa tcg act ata tct act tcc gca cgt tat tcg act gaa Pro Gln Ala Glu Ser Thr Ile Ser Thr Ser Ala Arg Tyr Ser Thr Glu 144 cgt cat aat ggt aat att aat aat att gaa tac gaa aat gtt agt tcg Arg His Asn Gly Asn Ile Asn Asn Ile Glu Tyr Glu Asn Val Ser Ser 35 192 ttg aaa gtt caa aaa ggg gca gct tct gta atg tat ggt agc ggt gcg Leu Lys Val Gln Lys Gly Ala Ala Ser Val Met Tyr Gly Ser Gly Ala 50 tta ggt gga acc gtg gag ttt acc aca aaa gat att gag gac ttt gtc 240 Leu Gly Gly Thr Val Glu Phe Thr Thr Lys Asp Ile Glu Asp Phe Val 65 70 gaa cct ggt cgc cat ttg ggc ttt ttg tct aaa acc ggc tat act tca 288 Glu Pro Gly Arg His Leu Gly Phe Leu Ser Lys Thr Gly Tyr Thr Ser 85 336 aaa aac aga gaa tat cgt caa gtc atc gga gtt gga ggg aaa ggg gaa Lys Asn Arg Glu Tyr Arg Gln Val Ile Gly Val Gly Gly Lys Gly Glu 100 384 cac ttt ttt ggt ttt gta caa tta acc aaa cgt tgg ggg cat gaa aca His Phe Phe Gly Phe Val Gln Leu Thr Lys Arg Trp Gly His Glu Thr 120

atc aac aac ggc aaa ggt aca gac att ctc ggc gaa cat cga ggt aaa

Ile	Asn 130	Asn	Gly	Lys	Gly	Thr 135	Asp	Ile	Leu	Gly	Glu 140	His	Arg	Gly	Lys	
		_										acg Thr		_		480
												tta Leu				528
												ctt Leu				576
												tat Tyr 205				624
												aag Lys				672
												gag Glu				720
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aga Arg	cag Gln	aat Asn	ata Ile 260	gct Ala	cgg Arg	gga Gly	gaa Glu	ttt Phe 265	tca Ser	acg Thr	agt Ser	cct Pro	tta Leu 270	tat Tyr	tgg Trp	816
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												ccg Pro				912
												gaa Glu				960
												gac Asp				1008
	_	_					_					gat Asp				1056
												agg Arg 365				1104
												caa Gln				1152

	tat Tyr															1200
	ttt Phe		_			_	_	_				_	_			1248
	agt Ser												_	_		1296
	ctg Leu															1344
	agc Ser 450	-				_									_	1392
	gag Glu															1440
	tat Tyr															1488
_	gtg Val					_	_	_		_				_	_	1536
	gga Gly												_		_	1584
	ggt Gly 530															1632
	gtg Val															1680
	agc Ser															1728
	gaa Glu															1776
agt Ser	cca Pro	tcc Ser 595	tac Tyr	ttt Phe	gtt Val	gtt Val	gat Asp 600	ttt Phe	acg Thr	gjà aaa	caa Gln	gtt Val 605	aac Asn	ctc Leu	agt Ser	1824
	aat Asn 610															1872
	atg Met															1920

tcc cgt tct gtc cgt gct aac agc cca ggc att aat cgg ttt acc gca
Ser Arg Ser Val Arg Ala Asn Ser Pro Gly Ile Asn Arg Phe Thr Ala
645 650 655

cca aaa cgt aat ttt gct gcc tcg gtg gaa att cgt ttt ta
Pro Lys Arg Asn Phe Ala Ala Ser Val Glu Ile Arg Phe
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2009

1968

<210> 105

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<212> PRT

<213> Pasteurella multocida

<400> 105

. 3

Half Br. Carr

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Arg His Asn Gly Asn Ile Asn Asn Ile Glu Tyr Glu Asn Val Ser Ser 35 40 45

Leu Lys Val Gln Lys Gly Ala Ala Ser Val Met Tyr Gly Ser Gly Ala
50 60

Leu Gly Gly Thr Val Glu Phe Thr Thr Lys Asp Ile Glu Asp Phe Val 65 70 75 80

Glu Pro Gly Arg His Leu Gly Phe Leu Ser Lys Thr Gly Tyr Thr Ser

Lys Asn Arg Glu Tyr Arg Gln Val Ile Gly Val Gly Gly Lys Gly Glu 100 105 110

His Phe Phe Gly Phe Val Gln Leu Thr Lys Arg Trp Gly His Glu Thr 115 120 125

Ile Asn Asn Gly Lys Gly Thr Asp Ile Leu Gly Glu His Arg Gly Lys
130 135 140

Pro Asn Pro Leu Asn Tyr Tyr Thr Thr Ser Trp Leu Thr Lys Val Gly
145 150 155 160

Tyr Asp Ile Asn Asn Thr His Arg Phe Thr Leu Phe Leu Glu Asp Arg 165 170 175

Arg Glu Lys Lys Leu Thr Glu Glu Lys Thr Leu Gly Leu Ser Asp Ala

Val Arg Phe Ala Asn Asp Gln Thr Pro Tyr Leu Arg Tyr Gly Ile Glu 195 200 205

Tyr Arg Tyr Asn Gly Leu Ser Trp Leu Glu Thr Val Lys Leu Phe Leu 210 220

Ala Lys Gln Lys Ile Glu Gln Arg Ser Ala Leu Gln Glu Phe Asp Ile 225 230 235 240 Asn Asn Arg Asn Lys Leu Asp Ser Thr Met Ser Phe Val Tyr Leu Gln Arg Gln Asn Ile Ala Arg Gly Glu Phe Ser Thr Ser Pro Leu Tyr Trp 265 Gly Pro Ser Arg His Arg Leu Ser Ala Lys Phe Glu Phe Arg Asp Lys Phe Leu Glu Asn Met Asn Lys His Phe Thr Phe Arg Pro Trp Gln Ile Asn Arg Phe Arg Gln Gln Gly Arg Asn Asn Tyr Thr Glu Val Phe Pro Val Lys Ser Arg Glu Phe Ser Phe Ser Leu Met Asp Asp Ile Lys Ile Gly Glu Leu Leu His Leu Gly Leu Gly Gly Arg Trp Asp His Tyr Asn Tyr Lys Pro Leu Leu Asn Ser Gln His Asn Ile Asn Arg Thr Gln Arg Leu Pro Tyr Pro Lys Thr Ser Ser Lys Phe Ser Tyr Gln Leu Ser Leu 375 Glu Tyr Gln Leu His Pro Ser His Gln Ile Ala Tyr Arg Leu Ser Thr 390 Gly Phe Arg Val Pro Arg Val Glu Asp Leu Tyr Phe Glu Asp Arg Gly 410 Lys Ser Ser Ser Gln Phe Leu Pro Asn Pro Asp Leu Gln Pro Glu Thr Ala Leu Asn His Glu Ile Ser Tyr Arg Phe Gln Asn Gln Tyr Ala His Phe Ser Val Gly Leu Phe Arg Thr Arg Tyr His Asn Phe Ile Gln Glu Arg Glu Met Thr Cys Asp Lys Ile Pro Tyr Glu Tyr Asn Arg Thr Tyr 470 Gly Tyr Cys Thr His Asn Thr Tyr Val Met Phe Val Asn Glu Pro Glu 490 Ala Val Ile Lys Gly Val Glu Val Ser Gly Ala Leu Asn Gly Ser Ala Phe Gly Leu Ser Asp Gly Leu Thr Phe Arg Leu Lys Gly Ser Tyr Ser 520 Lys Gly Gln Asn His Asp Gly Asp Pro Leu Lys Ser Ile Gln Pro Trp Thr Val Val Thr Gly Ile Asp Tyr Glu Thr Glu Gly Trp Ser Val Ser 545

570

Leu Ser Gly Arg Tyr Ser Ala Ala Lys Lys Ala Lys Asp Ala Ile Glu

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Thr Glu Tyr Thr His Asp Lys Lys Val Val Lys Gln Trp Pro His Leu
Ser Pro Ser Tyr Phe Val Val Asp Phe Thr Gly Gln Val Asn Leu Ser
Lys Asn Val Ile Leu Asn Met Gly Val Phe Asn Leu Phe Asn Arg Asp
                       615
Tyr Met Thr Trp Asp Ser Ala Tyr Asn Leu Phe Thr Arg Gly Tyr Thr
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gtt gcg att aaa agc att ata aat cat aat gaa aaa ggt att tca ttt
                                                                96
Val Ala Ile Lys Ser Ile Ile Asn His Asn Glu Lys Gly Ile Ser Phe
tat att ttt gat ttg ggt ata aag gat gaa aat aag aga aat att aat
                                                                144
Tyr Ile Phe Asp Leu Gly Ile Lys Asp Glu Asn Lys Arg Asn Ile Asn
gat att gtt tct tct tat gga agt gaa gtc aac ttt att gct gtg aat
                                                                192
Asp Ile Val Ser Ser Tyr Gly Ser Glu Val Asn Phe Ile Ala Val Asn
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gag aaa gaa ttt gag agt ttt cct gtt caa att agt tat att tct tta
Glu Lys Glu Phe Glu Ser Phe Pro Val Gln Ile Ser Tyr Ile Ser Leu
gca aca tat gca agg cta aaa gcg gca gag tat ttg ccg gat aat tta
                                                                288
Ala Thr Tyr Ala Arg Leu Lys Ala Ala Glu Tyr Leu Pro Asp Asn Leu
aat aaa att att tat tta gat gtt gat gtt ttg gtt ttt aac tca tta
Asn Lys Ile Ile Tyr Leu Asp Val Asp Val Leu Val Phe Asn Ser Leu
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Glu Met Leu Trp Asn Val Asp Val Asn Asn Phe Leu Thr Ala Ala Cys
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115 120 125

	gat Asp 130															432
	atg Met															480
	tta Leu															528
	tta Leu															576
	aat Asn															624
	ttc Phe 210	-					-	_							_	672
	ttg Leu															720
	tca Ser															768
	ttt Phe															816
ggc Gly	acg Thr	gat Asp 275	aaa Lys	gaa Glu	cgc Arg	gta Val	tta Leu 280	tct Ser	ata Ile	aaa Lys	act Thr	tat Tyr 285	ctc Leu	aag Lys	gcc Ala	864
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<211> 302

<212> PRT

<213> Pasteurella multocida

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Val Ala Ile Lys Ser Ile Ile Asn His Asn Glu Lys Gly Ile Ser Phe 20 30

Tyr Ile Phe Asp Leu Gly Ile Lys Asp Glu Asn Lys Arg Asn Ile Asn 35 40 45

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Asp Ile Val Ser Ser Tyr Gly Ser Glu Val Asn Phe Ile Ala Val Asn 50 55 60
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Glu Lys Glu Phe Glu Ser Phe Pro Val Gln Ile Ser Tyr Ile Ser Leu 65 70 75 80

Ala Thr Tyr Ala Arg Leu Lys Ala Ala Glu Tyr Leu Pro Asp Asn Leu 85 90 95

Asn Lys Ile Ile Tyr Leu Asp Val Asp Val Leu Val Phe Asn Ser Leu
100 105 110

Glu Met Leu Trp Asn Val Asp Val Asn Asn Phe Leu Thr Ala Ala Cys 115 120 125

Tyr Asp Ser Phe Ile Glu Asn Glu Lys Ser Glu His Lys Lys Ser Ile 130 135 140

Ser Met Ser Asp Lys Glu Tyr Tyr Phe Asn Ala Gly Val Met Leu Phe 145 150 155 160

Asn Leu Asp Glu Trp Arg Lys Met Asp Val Phe Ser Arg Ala Leu Asp 165 170 175

Leu Leu Ala Met Tyr Pro Asn Gln Met Ile Tyr Gln Asp Gln Asp Ile 180 185 190

Leu Asn Ile Leu Phe Arg Asn Lys Val Cys Tyr Leu Asp Cys Arg Phe
195 200 205

Asn Phe Met Pro Asn Gln Leu Glu Arg Ile Lys Gln Tyr His Lys Gly 210 215 220

Lys Leu Ser Asn Leu His Ser Leu Glu Lys Thr Thr Met Pro Val Val 225 230 235 240

Ile Ser His Tyr Cys Gly Pro Glu Lys Ala Trp His Ala Asp Cys Lys 245 250 255

His Phe Asn Val Tyr Phe Tyr Gln Lys Ile Leu Ala Glu Ile Thr Arg 260 265 270

Gly Thr Asp Lys Glu Arg Val Leu Ser Ile Lys Thr Tyr Leu Lys Ala 275 280 285

Leu Ile Arg Arg Ile Arg Tyr Lys Phe Lys Tyr Gln Val Tyr 290 295 300

<210> 108

<211> 2054

<212> DNA

<213> Pasteurella multocida

<220>

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<220>

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aaa Lys	gat Asp	gtg Val	aaa Lys 20	gaa Glu	ggt Gly	caa Gln	gac Asp	ttc Phe 25	ttc Phe	cca Pro	tta Leu	act Thr	gtt Val 30	aac Asn	tat Tyr	96
caa Gln	gag Glu	cgt Arg 35	act Thr	tat Tyr	gct Ala	gca Ala	ggc Gly 40	cgt Arg	att Ile	cct Pro	ggt Gly	ggc Gly 45	ttt Phe	ttc Phe	aaa Lys	144
cgt Arg	gaa Glu 50	ggt Gly	cgt Arg	cct Pro	tct Ser	gaa Glu 55	ggc Gly	gaa Glu	act Thr	tta Leu	att Ile 60	gct Ala	cgt Arg	tta Leu	att Ile	192
gac Asp 65	cgt Arg	cca Pro	att Ile	cgt Arg	cct Pro 70	ctt Leu	ttc Phe	cca Pro	gaa Glu	ggt Gly 75	ttt Phe	tat Tyr	aac Asn	gaa Glu	atc Ile 80	240
caa Gln	atc Ile	gtg Val	gcg Ala	aca Thr 85	gtg Val	gtg Val	tct Ser	gtt Val	aat Asn 90	ccg Pro	caa Gln	att Ile	tgt Cys	cca Pro 95	gat Asp	288
tta Leu	gtg Val	gca Ala	atg Met 100	atc Ile	ggt Gly	gca Ala	tct Ser	gcg Ala 105	gca Ala	ctt Leu	tct Ser	tta Leu	tca Ser 110	ggt Gly	gtg Val	336
cca Pro	ttt Phe	aat Asn 115	ggc Gly	cct Pro	atc Ile	ggt Gly	gcg Ala 120	gca Ala	cgt Arg	gtt Val	ggt Gly	ttt Phe 125	att Ile	gat Asp	gat Asp	384
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ggt Gly	cat His	cag Gln	caa Gln 180	caa Gln	caa Gln	gtg Val	gtg Val	att Ile 185	gac Asp	gcg Ala	atc Ile	aaa Lys	gaa Glu 190	ttt Phe	acc Thr	576
gca Ala	gaa Glu	gcc Ala 195	ggt Gly	aaa Lys	ccg Pro	cgt Arg	tgg Trp 200	gat Asp	tgg Trp	gtg Val	gca Ala	cct Pro 205	gaa Glu	cca Pro	aat Asn	624
acc Thr	gcg Ala 210	tta Leu	att Ile	gaa Glu	aaa Lys	gtg Val 215	aaa Lys	gcg Ala	att Ile	gca Ala	gaa Glu 220	gcg Ala	cgt Arg	tta Leu	ggc Gly	672
												gaa Glu				720
												gaa Glu				768

245 250 255

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gaa ag Glu Se	c caa r Gln 275	atc Ile	gta Val	cgt Arg	agc Ser	cgt Arg 280	atc Ile	att Ile	gct Ala	ggt Gly	gaa Glu 285	cca Pro	cgt Arg	att Ile	864
gat gg Asp Gl 29	y Arg	aca Thr	gtg Val	gat Asp	act Thr 295	gtt Val	cgt Arg	gca Ala	tta Leu	gat Asp 300	att Ile	tgt Cys	act Thr	ggt Gly	912
gtt tt: Val Le 305	a cca ı Pro	cgt Arg	aca Thr	cac His 310	ggt Gly	tct Ser	gcg Ala	att Ile	ttc Phe 315	acc Thr	cgt Arg	ggt Gly	gaa Glu	aca Thr 320	960
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ggt gc Gly Al															1296
gtt gc Val Al	a ggt a Gly 435	att Ile	gca Ala	atg Met	ggc Gly	tta Leu 440	gtc Val	aaa Lys	gaa Glu	gac Asp	gaa Glu 445	aaa Lys	ttt Phe	gtg Val	1344
gtg ct Val Le 45	ı Ser	gac Asp	atc Ile	tta Leu	ggt Gly 455	gat Asp	gaa Glu	gat Asp	cac His	tta Leu 460	ggt Gly	gac Asp	atg Met	gac Asp	1392
ttc aa Phe Ly: 465															1440
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caa Gln	gcg Ala	aaa Lys	agc Ser 500	gca Ala	cgt Arg	tta Leu	cac His	att Ile 505	tta Leu	ggt Gly	gtg Val	atg Met	gag Glu 510	caa Gln	gcg Ala	1536
atc Ile	cca Pro	gcg Ala 515	cca Pro	cgt Arg	gcg Ala	gat Asp	att Ile 520	tct Ser	gat Asp	ttt Phe	gca Ala	ccg Pro 525	cgt Arg	att Ile	tac Tyr	1584
act Thr	atg Met 530	aaa Lys	att Ile	gat Asp	ccg Pro	aag Lys 535	aaa Lys	atc Ile	aaa Lys	gat Asp	gtg Val 540	atc Ile	ggt Gly	aaa Lys	ggt Gly	1632
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gcg Ala	ggt Gly	gca Ala 595	gtg Val	tat Tyr	aaa Lys	ggt Gly	aaa Lys 600	gtt Val	act Thr	cgt Arg	tta Leu	gct Ala 605	gat Asp	ttt Phe	ggt Gly	1824
gcc Ala	ttc Phe 610	gtt Val	tct Ser	atc Ile	gta Val	ggt Gly 615	aac Asn	aaa Lys	gaa Glu	ggc	tta Leu 620	gtg Val	cat His	att Ile	tct Ser	1872
caa Gln 625	atc Ile	gcg Ala	gaa Glu	gag Glu	cgt Arg 630	gtt Val	gag Glu	aaa Lys	gtg Val	agt Ser 635	gat Asp	tat Tyr	ctt Leu	gca Ala	gtg Val 640	1920
gly ggg	caa Gln	gaa Glu	gtg Val	act Thr 645	gtt Val	aaa Lys	gtg Val	gtt Val	gag Glu 650	att Ile	gat Asp	cgt Arg	caa Gln	ggt Gly 655	cgt Arg	1968
att Ile	cgt Arg	tta Leu	acc Thr 660	Met	aaa Lys	gaa Glu	gtt Val	gca Ala 665	cca Pro	aag Lys	caa Gln	gaa Glu	cac His 670	gtt Val	gat Asp	2016
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<211> 684

<212> PRT

<213> Pasteurella multocida

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Gln Glu Arg Thr Tyr Ala Ala Gly Arg Ile Pro Gly Gly Phe Phe Lys

35 40 45

Arg	Glu 50	Gly	Arg	Pro	Ser	Glu 55	Gly	Glu	Thr	Leu	Ile 60	Ala	Arg	Leu	Ile
Asp 65	Arg	Pro	Ile	Arg	Pro 70	Leu	Phe	Pro	Glu	Gly 75	Phe	Tyr	Asn	Glu	Ile 80
Gln	Ile	Val	Ala	Thr 85	Val	Val	Ser	Val	Asn 90	Pro	Gln	Ile	Cys	Pro 95	Asp
Leu	Val	Ala	Met 100	Ile	Gly	Ala	Ser	Ala 105	Ala	Leu	Ser	Leu	Ser 110	Gly	Val
Pro	Phe	Asn 115	Gly	Pro	Ile	Gly	Ala 120	Ala	Arg	Val	Gly	Phe 125	Ile	Asp	Asp
Gln	Phe 130	Val	Leu	Asn	Pro	Thr 135	Met	Asn	Glu	Gln	Lys 140	Gln	Ser	Arg	Leu
Asp 145	Leu	Val	Val	Ala	Gly 150	Thr	Asp	Lys	Ala	Val 155	Leu	Met	Val	Glu	Ser 160
				165					170					Val 175	
			180					185					190	Phe	
		195					200					205		Pro	
	210					215					220			Leu	
225					230					235				Ile	240
				245					250					Ala 255	
			260					265					270	Ala	
		275					280					285		Arg	
	290					295					300			Thr	
305					310					315				Glu	320
				325					330					Gln 335	
			340					345					350		
Asn	Phe	Pro 355		Tyr	Ser	Val	Gly 360	Glu	Thr	Gly	Met	Ile 365		Ser	Pro

Lys Arg Arg Glu Ile Gly His Gly Arg Leu Ala Lys Arg Gly Val Ala Ala Val Met Pro Thr Leu Ala Glu Phe Pro Tyr Val Val Arg Val Val 390 Ser Glu Ile Thr Glu Ser Asn Gly Ser Ser Ser Met Ala Ser Val Cys 410 Gly Ala Ser Leu Ala Leu Met Asp Ala Gly Val Pro Ile Lys Ala Ala 425 Val Ala Gly Ile Ala Met Gly Leu Val Lys Glu Asp Glu Lys Phe Val 440 Val Leu Ser Asp Ile Leu Gly Asp Glu Asp His Leu Gly Asp Met Asp Phe Lys Val Ala Gly Thr Arg Thr Gly Val Thr Ala Leu Gln Met Asp Ile Lys Ile Glu Gly Ile Thr Ala Glu Ile Met Gln Ile Ala Leu Asn Gln Ala Lys Ser Ala Arg Leu His Ile Leu Gly Val Met Glu Gln Ala 505 Ile Pro Ala Pro Arg Ala Asp Ile Ser Asp Phe Ala Pro Arg Ile Tyr 520 Thr Met Lys Ile Asp Pro Lys Lys Ile Lys Asp Val Ile Gly Lys Gly Gly Ala Thr Ile Arg Ala Leu Thr Glu Glu Thr Gly Thr Ser Ile Asp Ile Asp Asp Asp Gly Thr Val Lys Ile Ala Ala Val Asp Gly Asn Ser 570 Ala Lys Glu Val Met Ala Arg Ile Glu Asp Ile Thr Ala Glu Val Glu Ala Gly Ala Val Tyr Lys Gly Lys Val Thr Arg Leu Ala Asp Phe Gly Ala Phe Val Ser Ile Val Gly Asn Lys Glu Gly Leu Val His Ile Ser Gln Ile Ala Glu Glu Arg Val Glu Lys Val Ser Asp Tyr Leu Ala Val 625 Gly Gln Glu Val Thr Val Lys Val Val Glu Ile Asp Arg Gln Gly Arg Ile Arg Leu Thr Met Lys Glu Val Ala Pro Lys Gln Glu His Val Asp Ser Val Val Ala Asp Val Ala Ala Glu Glu Asn Ala

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att tat gat gcg tta acc tta ttg caa cac cgc ggg caa gac gcc gcc
Ile Tyr Asp Ala Leu Thr Leu Leu Gln His Arg Gly Gln Asp Ala Ala
ggg att gta acc gta gat gat gaa aac cga ttc cgc ttg cgt aaa gcg
                                                                   144
Gly Ile Val Thr Val Asp Asp Glu Asn Arg Phe Arg Leu Arg Lys Ala
         35
aac ggg tta gtc agc gat gta ttt gaa caa gtt cat atg tta cgt tta
                                                                   192
Asn Gly Leu Val Ser Asp Val Phe Glu Gln Val His Met Leu Arg Leu
caa ggc aat gct ggc att gga cat gtt cgt tat cct acg gct ggg agc
Gln Gly Asn Ala Gly Ile Gly His Val Arg Tyr Pro Thr Ala Gly Ser
tca agt gtc tct gaa gcg caa cct ttt tat gta aat tcg cct tat ggc
                                                                   288
Ser Ser Val Ser Glu Ala Gln Pro Phe Tyr Val Asn Ser Pro Tyr Gly
                 85
                                                                   336
tta acc tta gtg cat aat ggt aac ttg acc aat tca agt gaa tta aaa
Leu Thr Leu Val His Asn Gly Asn Leu Thr Asn Ser Ser Glu Leu Lys
            100
gaa aag tta ttt cgt ctc gca cgt cgc cat gta aat acc aat tca gat
                                                                   384
Glu Lys Leu Phe Arg Leu Ala Arg Arg His Val Asn Thr Asn Ser Asp
                            120
                                                                   432
tot gaa tta tta oto aat ato tta goo aat cao ott gat cao tto gaa
Ser Glu Leu Leu Asn Ile Leu Ala Asn His Leu Asp His Phe Glu
                        135
aaa tac caa tta gat ccg caa gat gta ttc agt gct gtc aaa caa acg
Lys Tyr Gln Leu Asp Pro Gln Asp Val Phe Ser Ala Val Lys Gln Thr
                    150
                                        155
cat cag gat att cgt ggt gct tat gct tgt atc gcc atg att att ggt
                                                                   528
His Gln Asp Ile Arg Gly Ala Tyr Ala Cys Ile Ala Met Ile Ile Gly
cat ggt atg gtc gcg ttt cgt gat ccg aac ggt atc cgt ccg tta gtg
                                                                   576
His Gly Met Val Ala Phe Arg Asp Pro Asn Gly Ile Arg Pro Leu Val
tta ggg aaa cgc gag gaa aat ggc aaa aca gag tat atg ttt gcc tcc
Leu Gly Lys Arg Glu Glu Asn Gly Lys Thr Glu Tyr Met Phe Ala Ser
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195 200 205

gaa Glu	agt Ser 210	atc Ile	gca Ala	tta Leu	gat Asp	aca Thr 215	gtg Val	ggt Gly	ttt Phe	gag Glu	ttt Phe 220	gta Val	cga Arg	gat Asp	gta Val	672
caa Gln 225	ccc Pro	ggc Gly	gaa Glu	gcg Ala	att Ile 230	tat Tyr	gtc Val	acg Thr	ttt Phe	gaa Glu 235	gl <sup>y</sup> aaa	gaa Glu	atg Met	tat Tyr	gct Ala 240	720
cag Gln	caa Gln	tgc Cys	gca Ala	gac Asp 245	aaa Lys	cca Pro	aca Thr	tta Leu	aca Thr 250	cct Pro	tgt Cys	att Ile	ttt Phe	gaa Glu 255	tac Tyr	768
gtc Val	tat Tyr	ttt Phe	gca Ala 260	cgt Arg	cca Pro	gac Asp	tct Ser	tgc Cys 265	atc Ile	gat Asp	ggg Gly	gtt Val	tct Ser 270	gtt Val	tat Tyr	816
gct Ala	gcc Ala	cgt Arg 275	gtt Val	cat His	atg Met	gga Gly	caa Gln 280	cgt Arg	tta Leu	ggt Gly	gaa Glu	aaa Lys 285	att Ile	gca Ala	cgg Arg	864
gaa Glu	tgg Trp 290	gcg Ala	gat Asp	gtg Val	gat Asp	gat Asp 295	att Ile	gat Asp	gtg Val	gtc Val	att Ile 300	cct Pro	gtg Val	cct Pro	gaa Glu	912
acc Thr 305	tct Ser	aac Asn	gat Asp	att Ile	gct Ala 310	tta Leu	cgt Arg	att Ile	gcg Ala	cgc Arg 315	gtg Val	tta Leu	aat Asn	aaa Lys	ccg Pro 320	960
tat Tyr	cgt Arg	caa Gln	ggt Gly	ttt Phe 325	gtg Val	aaa Lys	aat Asn	cgc Arg	tat Tyr 330	gta Val	gga Gly	cgt Arg	acg Thr	ttt Phe 335	att Ile	1008
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acc Thr	att Ile	gct Ala 355	tca Ser	gaa Glu	ttt Phe	aaa Lys	gat Asp 360	aag Lys	aat Asn	gtg Val	tta Leu	tta Leu 365	gtt Val	gac Asp	gac Asp	1104
tcg Ser	att Ile 370	gta Val	cgt Arg	ggt Gly	acc Thr	acg Thr 375	tct Ser	gaa Glu	caa Gln	att Ile	gtc Val 380	GIu	atg Met	gcg Ala	aga Arg	1152
gcg Ala 385	Ala	ggt Gly	gcg Ala	aag Lys	aaa Lys 390	att Ile	tat Tyr	ttt Phe	gcc Ala	tct Ser 395	· Ala	gca Ala	cca Pro	gaa Glu	att Ile 400	1200
cgt Arg	tat Tyr	cca Pro	aat Asn	gtg Val 405	tat Tyr	ggt Gly	att	gat Asp	atg Met 410	Pro	acc Thr	aaa Lys	aat Asn	gaa Glu 415	ttg Leu	1248
atc Ile	gct Ala	tat Tyr	ggt Gly 420	cgt Arg	gat Asp	gta Val	gat Asp	gaa Glu 425	Ile	gct Ala	aac Asn	tta Leu	att Ile 430	Gly	gtg Val	1296
gat Asp	aaa Lys	ttg Leu 435	Ile	ttc Phe	caa Gln	gat Asp	ttg Leu 440	. Asp	gcg Ala	j tta Leu	act Thr	ggt Gly 445	Ser	gtg Val	caa Gln	1344

115

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Lys Tyr Gln Leu Asp Pro Gln Asp Val Phe Ser Ala Val Lys Gln Thr

Ser Glu Leu Leu Asn Ile Leu Ala Asn His Leu Asp His Phe Glu

Glu Lys Leu Phe Arg Leu Ala Arg Arg His Val Asn Thr Asn Ser Asp

Lys Tyr Gln Leu Asp Pro Gln Asp Val Phe Ser Ala Val Lys Gln Thr 145 150 155

His Gln Asp Ile Arg Gly Ala Tyr Ala Cys Ile Ala Met Ile Ile Gly 165 170 175

His Gly Met Val Ala Phe Arg Asp Pro Asn Gly Ile Arg Pro Leu Val 180 185 190

Leu Gly Lys Arg Glu Glu Asn Gly Lys Thr Glu Tyr Met Phe Ala Ser 195 200 205

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Glu Ser Ile Ala Leu Asp Thr Val Gly Phe Glu Phe Val Arg Asp Val
Gln Pro Gly Glu Ala Ile Tyr Val Thr Phe Glu Gly Glu Met Tyr Ala
Gln Gln Cys Ala Asp Lys Pro Thr Leu Thr Pro Cys Ile Phe Glu Tyr
                                    250
Val Tyr Phe Ala Arg Pro Asp Ser Cys Ile Asp Gly Val Ser Val Tyr
Ala Ala Arg Val His Met Gly Gln Arg Leu Gly Glu Lys Ile Ala Arg
Glu Trp Ala Asp Val Asp Asp Ile Asp Val Val Ile Pro Val Pro Glu
Thr Ser Asn Asp Ile Ala Leu Arg Ile Ala Arg Val Leu Asn Lys Pro
Tyr Arg Gln Gly Phe Val Lys Asn Arg Tyr Val Gly Arg Thr Phe Ile
Met Pro Gly Gln Ala Leu Arg Val Ser Ser Val Arg Arg Lys Leu Asn
Thr Ile Ala Ser Glu Phe Lys Asp Lys Asn Val Leu Leu Val Asp Asp
Ser Ile Val Arg Gly Thr Thr Ser Glu Gln Ile Val Glu Met Ala Arg
Ala Ala Gly Ala Lys Lys Ile Tyr Phe Ala Ser Ala Ala Pro Glu Ile
Arg Tyr Pro Asn Val Tyr Gly Ile Asp Met Pro Thr Lys Asn Glu Leu
                                    410
Ile Ala Tyr Gly Arg Asp Val Asp Glu Ile Ala Asn Leu Ile Gly Val
Asp Lys Leu Ile Phe Gln Asp Leu Asp Ala Leu Thr Gly Ser Val Gln
Gln Glu Asn Pro Ser Ile Gln Asp Phe Asp Cys Ser Val Phe Thr Gly
Val Tyr Val Thr Gly Asp Ile Thr Pro Glu Tyr Leu Asp Asn Ile Ala
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Glu Gln Arg Asn Asp Ile Ala Lys Lys Lys Arg Glu Lys Asp Ala Thr

Asn Leu Glu Met His Asn Glu Lys 500

490

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<sup>&</sup>lt;211> 989

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210 215 220

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cta att tta Leu Ile Leu	aaa atg Lys Met 245	aaa g Lys G	gag gt Glu Va	a gaa l Glu	aat Asn 250	gga Gly	gat Asp	ctt Leu	gtg Val	ttt Phe 255	cag Gln	768
acc acg cct Thr Thr Pro	gaa tca Glu Ser 260	tta a Leu S	agc ac Ser Th	c acg r Thr 265	ttt Phe	aga Arg	gtg Val	tta Leu	aag Lys 270	aaa Lys	gag Glu	816
tgt gga ctt Cys Gly Leu 275	gaa cat Glu His	ctc c Leu F	cat tt His Ph 28	e His	gat Asp	acg Thr	aga Arg	agg Arg 285	gaa Glu	gcg Ala	ttg Leu	864
acg aga tta Thr Arg Leu 290	tct aag Ser Lys	Lys V	gta ga Val As 295	t gta p Val	atg Met	act Thr	cta Leu 300	gcc Ala	aaa Lys	att Ile	agc Ser	912
gga cat aga Gly His Arg 305	gat tta Asp Leu	aga a Arg I 310	att tt Ile Le	a caa u Gln	aac Asn	aca Thr 315	tat Tyr	tac Tyr	gca Ala	ccg Pro	aat Asn 320	960
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130					135					140				
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Ser Glu	Gln	Asp	Ile 165	Lys	Thr	Ile	Leu	Glu 170	Thr	Ala	Arg	Tyr	Cys 175	Glu
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Phe Ala	Ile 195	Glu	Thr	Ala	Met	Arg 200	Ala	Gly	Glu	Ile	Ala 205	Ser	Ile	Lys
Trp Asp		Val	Phe	Leu	Glu 215	Lys	Arg	Ile	Val	His 220	Leu	Pro	Thr	Thr
Lys Asr 225	Gly	His	Ser	Arg 230	Asp	Val	Pro	Leu	Ser 235	Gln	Arg	Ala	Val	Ala 240
Leu Ile	e Leu	Lys	Met 245	Lys	Glu	Val	Glu	Asn 250	Gly	Asp	Leu	Val	Phe 255	Gln
Thr Thr	Pro	Glu 260	Ser	Leu	Ser	Thr	Thr 265	Phe	Arg	Val	Leu	Lys 270	Lys	Glu

Cys Gly Leu Glu His Leu His Phe His Asp Thr Arg Arg Glu Ala Leu

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Ala Ile Arg Thr Ile Gln Ser Leu Ser Thr Ala Val Ile Gly Ile Val
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Cys Thr Ala Asn Asp Ala Asp Asn Glu Thr Phe Pro Leu Asn Glu Pro

35 40 45

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			_	_	_			_						aca Thr		336
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														gtg Val 255		768
														ggc Gly		816
														aag Lys		864

Glu V	-			_	act Thr	_					_	_		_		912
gcg t Ala P 305																960
gat a Asp I																1008
ggc t Gly T																1056
gca a Ala T	hr															1104
cca g Pro V 3			_		-	-					_				_	1152
gaa t Glu T 385			_	_				_		_	_	ta				1190
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<pre>&lt;212&gt; &lt;213&gt; &lt;400&gt; Met S     1  Ala I  Cys T  Val L  Gly T     65  Lys V</pre>	Pa 11 der le thr eu 50 thr al	steu  5 Glu  Arg  Ala 35 Ile  Leu  Ile  Ala	Glu Thr 20 Asn Thr Ser Val Ser 100	Tyr 5 Ile Asp Asn Arg Val 85 Glu	Leu Gln Ala Val Ala 70 Arg	His Ser Asp Ala 55 Leu Val	Leu Asn 40 Ala Asp Gln Thr	Ser 25 Glu Ala Gly Glu Ala 105	Thr Thr Ile Ile Ser 90 Ile	Ala Phe Gly Ser 75 Ala Ile	Val Pro Lys 60 Asp Gln	Ile Leu 45 Ala Val Glu Thr	Gly 30 Asn Gly Val Asp Ile 110	15 Ile Glu Lys Asn Glu 95 Thr	Val Pro Gln Cys 80 Glu Glu	

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Glu Val Ala Thr Glu Leu Ala Ser Ile Ala Ala Lys Leu Asn Ala Phe
145
Ala Tyr Ile Ser Cys Gln Gly Cys Lys Thr Lys Glu Gln Ala Val Gln
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Tyr Lys Arg Asn Phe Ser Gln Arg Glu Val Met Leu Ile Met Gly Asp
            180
Phe Leu Ser Phe Asn Val Asn Thr Ser Lys Val Glu Ile Asp Tyr Ala
                            200
Val Thr Arg Ala Ala Ala Met Arg Ala Tyr Leu Asp Lys Glu Gln Gly
Trp His Thr Ser Ile Ser Asn Lys Gly Ile Asn Gly Val Ser Gly Val
                    230
Thr Gln Pro Leu Tyr Phe Asp Ile Asn Asp Ser Ser Thr Asp Val Asn
Tyr Leu Asn Glu Gln Gly Ile Thr Cys Cys Val Asn His Asn Gly Phe
Arg Phe Trp Gly Leu Arg Thr Thr Ala Glu Asp Pro Leu Phe Lys Phe
                            280
Glu Val Tyr Thr Arg Thr Ala Gln Ile Leu Lys Asp Thr Ile Ala Gly
Ala Phe Asp Trp Ala Val Asp Lys Asp Ile Ser Val Thr Leu Val Lys
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                                        315
Asp Ile Ile Glu Ala Ile Asn Ala Lys Trp Arg Asp Tyr Thr Thr Lys
Gly Tyr Leu Ile Gly Gly Lys Ala Trp Leu Asn Lys Glu Leu Asn Ser
Ala Thr Asn Leu Lys Asp Ala Lys Leu Leu Ile Ser Tyr Asp Tyr His
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<400> 116

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ctt Leu	gta Val	cct Pro	gtt Val 20	gct Ala	gaa Glu	acg Thr	att Ile	aat Asn 25	tct Ser	gca Ala	gta Val	gga Gly	aat Asn 30	gcc Ala	tca Ser	96
tca Ser	aaa Lys	gac Asp 35	gtt Val	tct Ser	gac Asp	acc Thr	gag Glu 40	ata Ile	agt Ser	gct Ala	tct Ser	caa Gln 45	cca Pro	gcg Ala	ctc Leu	144
aac Asn	tcg Ser 50	ccg Pro	ctt Leu	tcg Ser	acc Thr	ctt Leu 55	tct Ser	gta Val	tta Leu	gtc Val	aaa Lys 60	acc Thr	gca Ala	ttt Phe	aat Asn	192
ccg Pro 65	gtt Val	tca Ser	aca Thr	ttg Leu	atg Met 70	tcg Ser	ttg Leu	act Thr	tgg Trp	aaa Lys 75	gaa Glu	tac Tyr	gcc Ala	gtt Val	tta Leu 80	240
tta Leu	tta Leu	agt Ser	gtg Val	gtg Val 85	tct Ser	ttt Phe	cct Pro	ctt Leu	atg Met 90	gca Ala	caa Gln	gcc Ala	tct Ser	gat Asp 95	aca Thr	288
gat Asp	tca Ser	gtg Val	gta Val 100	caa Gln	aga Arg	aaa Lys	cct Pro	gaa Glu 105	tta Leu	act Thr	gat Asp	gtg Val	acg Thr 110	aat Asn	agc Ser	336
aac Asn	agc Ser	tat Tyr 115	cat His	gtg Val	gaa Glu	tta Leu	gat Asp 120	aga Arg	gag Glu	cat His	cat His	aaa Lys 125	Gly aaa	gag Glu	cat His	384
caa Gln	aca Thr 130	aaa Lys	atc Ile	aaa Lys	cat His	act Thr 135	gag Glu	aat Asn	aat Asn	gtc Val	atc Ile 140	att Ile	gtt Val	gat Asp	att Ile	432
gca Ala 145	aaa Lys	cca Pro	aac Asn	caa Gln	aag Lys 150	ggc Gly	att Ile	tca Ser	gat Asp	aac Asn 155	cgt Arg	ttt Phe	aaa Lys	cac His	ttc Phe 160	480
aac Asn	atc Ile	cca Pro	aat Asn	999 Gly 165	gcg Ala	gta Val	ttt Phe	aac Asn	aat Asn 170	agc Ser	gcc Ala	aag Lys	gaa Glu	aaa Lys 175	cgc Arg	528
tca Ser	cag Gln	tta Leu	gtg Val 180	gly aaa	tat Tyr	ttg Leu	cca Pro	ggt Gly 185	aac Asn	cag Gln	aat Asn	tta Leu	acg Thr 190	gaa Glu	ggt Gly	576
agt Ser	gaa Glu	gca Ala 195	aaa Lys	gcg Ala	atc Ile	tta Leu	aat Asn 200	cag Gln	gtg Val	act Thr	gga Gly	ccg Pro 205	gat Asp	gcc Ala	agt Ser	624
aaa Lys	att Ile 210	gaa Glu	ggc Gly	gcc Ala	ctt Leu	gaa Glu 215	att Ile	tta Leu	Gly 999	caa Gln	aaa Lys 220	gcc Ala	gat Asp	ttg Leu	gtg Val	672
att Ile 225	Ala	aac Asn	caa Gln	aat Asn	ggc Gly 230	att Ile	gtg Val	ctt Leu	aat Asn	999 Gly 235	gta Val	aaa Lys	acc Thr	att Ile	aat Asn 240	720
gcc Ala	aat Asn	cgt Arg	ttt Phe	gtg Val	gca Ala	aca Thr	acc Thr	agt Ser	agt Ser	acc Thr	att Ile	gat Asp	cct Pro	gag Glu	caa Gln	768

245 250 255

atg Met	cag Gln	tta Leu	aat Asn 260	gtc Val	acg Thr	caa Gln	ggt Gly	aca Thr 265	gtg Val	aca Thr	att Ile	Gly 999	gtg Val 270	gat Asp	gga Gly	816
ttt Phe	gcc Ala	aca Thr 275	gat Asp	ggc Gly	tta Leu	cct Pro	tat Tyr 280	ttg Leu	gat Asp	atc Ile	att Ile	gcc Ala 285	aaa Lys	aag Lys	att Ile	864
gaa Glu	caa Gln 290	aaa Lys	caa Gln	gcg Ala	att Ile	aca Thr 295	aaa Lys	gaa Glu	aga Arg	aca Thr	gga Gly 300	aat Asn	tcc Ser	gaa Glu	acc Thr	912
gat Asp 305	atc Ile	act Thr	ttt Phe	gtc Val	gca Ala 310	ggt Gly	aac Asn	agt Ser	aaa Lys	tat Tyr 315	gat Asp	tta Leu	aag Lys	aca Thr	cat His 320	960
caa Gln	gtg Val	aca Thr	gaa Glu	aag Lys 325	cat His	acc Thr	gct Ala	gag Glu	gca Ala 330	caa Gln	ggt Gly	gaa Glu	att Ile	gcg Ala 335	att Ile	1008
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gag Glu	gcg Ala 370	gat Asp	att Ile	caa Gln	att Ile	gaa Glu 375	acc Thr	cat His	gag Glu	ggc	gat Asp 380	gtt Val	gaa Glu	tta Leu	ggc Gly	1152
aat Asn 385	aca Thr	aaa Lys	aat Asn	aat Asn	cag Gln 390	aat Asn	gag Glu	aat Asn	tat Tyr	gcc Ala 395	aaa Lys	gct Ala	cat His	gcg Ala	gaa Glu 400	1200
Gly 333	aat Asn	ttt Phe	acg Thr	gtt Val 405	aaa Lys	ggc	ggt Gly	aag Lys	cac His 410	gtt Val	att Ile	att Ile	ggt Gly	aag Lys 415	gaa Glu	1248
gtt Val	aaa Lys	gcc Ala	aac Asn 420	aaa Lys	gcg Ala	gtc Val	gat Asp	att Ile 425	caa Gln	gca Ala	caa Gln	gaa Glu	aca Thr 430	Thr	gta Val	1296
aga Arg	caa Gln	aat Asn 435	Ala	aaa Lys	tta Leu	act Thr	gcc Ala 440	Lys	acg Thr	agt Ser	gcc Ala	aaa Lys 445	TTE	aca Thr	gca Ala	1344
agt Ser	aag Lys 450	Ser	gtg Val	aat Asn	ctt Leu	gaa Glu 455	Asp	aac Asn	gcg Ala	aaa Lys	ctt Leu 460	ıI⊥∈	gct Ala	aat Asr	gag Glu	1392
ctg Leu 465	Ser	aca Thr	aca Thr	acc Thr	aat Asn 470	aaa Lys	tta Leu	acc Thr	aat Asn	aaa Lys 475	Gly	ago Ser	att Ile	tac Tyr	ggc Gly 480	1440
aag Lys	aaa Lys	gtg Val	acg Thr	cta Leu 485	Asp	gct Ala	gat Asp	aat Asn	tta Leu 490	. Val	: aat . Asr	agt Ser	aaa Lys	gaa Glu 495	atc lle	1488

tat Tyr	gcg Ala	tct Ser	agc Ser 500	gaa Glu	ctt Leu	gat Asp	att Ile	caa Gln 505	acc Thr	aaa Lys	ggt Gly	cgt Arg	gat Asp 510	ctt Leu	tta Leu	1536
ctt Leu	gag Glu	gat Asp 515	gl <sup>y</sup> aaa	gtt Val	aat Asn	caa Gln	cca Pro 520	ctg Leu	agt Ser	ttc Phe	tta Leu	aaa Lys 525	ggc Gly	gct Ala	tca Ser	1584
ttg Leu	tta Leu 530	gcg Ala	ccg Pro	Gly aaa	ttt Phe	gtc Val 535	aac Asn	act Thr	gly ggg	cta Leu	att Ile 540	cac His	agt Ser	aac Asn	ggt Gly	1632
aat Asn 545	gcc Ala	aag Lys	ctc Leu	act Thr	ttt Phe 550	aaa Lys	gat Asp	gac Asp	acc Thr	agt Ser 555	ttt Phe	gtg Val	act Thr	gaa Glu	gga Gly 560	1680
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atc Ile	aat Asn	acc Thr 595	aag Lys	tct Ser	ggt Gly	ttt Phe	gtg Val 600	aat Asn	tac Tyr	ggt Gly	acc Thr	tta Leu 605	gca Ala	agt Ser	gct Ala	1824
caa Gln	aat Asn 610	tta Leu	acg Thr	att Ile	aat Asn	acc Thr 615	gaa Glu	caa Gln	ggc Gly	agc Ser	att Ile 620	tat Tyr	aac Asn	ata Ile	ggc Gly	1872
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gaa Glu	aac Asn	caa Gln	gga Gly	gga Gly 645	tat Tyr	ctt Leu	att Ile	aat Asn	caa Gln 650	ggt Gly	aag Lys	agt Ser	cta Leu	ctc Leu 655	cat His	1968
tct Ser	gaa Glu	ggc Gly	gcc Ala 660	atg Met	aac Asn	ctc Leu	aca Thr	gcg Ala 665	gat Aspi	cgc Arg	acg Thr	gtg Val	tac Tyr 670	aat Asn	tta Leu	2016
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aaa Lys 705	gat Asp	tat Tyr	acg Thr	cgt Arg	tat Tyr 710	tat Tyr	cgt Arg	atc Ile	aat Asn	gaa Glu 715	acg Thr	gca Ala	aaa Lys	cat His	ggt Gly 720	2160
tgg Trp	cat His	aat Asn	aac Asn	ttc Phe 725	tat Tyr	gaa Glu	tta Leu	aac Asn	gtc Val 730	gac Asp	aga Arg	gtt Val	tct Ser	tg		2204

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<212> PRT

<213> Pasteurella multocida

<400> 117

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Ser Lys Asp Val Ser Asp Thr Glu Ile Ser Ala Ser Gln Pro Ala Leu 35 40 45

Asn Ser Pro Leu Ser Thr Leu Ser Val Leu Val Lys Thr Ala Phe Asn 50 55 60

Pro Val Ser Thr Leu Met Ser Leu Thr Trp Lys Glu Tyr Ala Val Leu 65 70 75 80

Leu Leu Ser Val Val Ser Phe Pro Leu Met Ala Gln Ala Ser Asp Thr 85 90 95

Asp Ser Val Val Gln Arg Lys Pro Glu Leu Thr Asp Val Thr Asn Ser 100 105 110

Asn Ser Tyr His Val Glu Leu Asp Arg Glu His His Lys Gly Glu His 115 120 125

Gln Thr Lys Ile Lys His Thr Glu Asn Asn Val Ile Ile Val Asp Ile 130 135 140

Ala Lys Pro Asn Gln Lys Gly Ile Ser Asp Asn Arg Phe Lys His Phe 145 150 155 160

Asn Ile Pro Asn Gly Ala Val Phe Asn Asn Ser Ala Lys Glu Lys Arg 165 170 175

Ser Gln Leu Val Gly Tyr Leu Pro Gly Asn Gln Asn Leu Thr Glu Gly 180 185 190

Ser Glu Ala Lys Ala Ile Leu Asn Gln Val Thr Gly Pro Asp Ala Ser 195 200 205

Lys Ile Glu Gly Ala Leu Glu Ile Leu Gly Gln Lys Ala Asp Leu Val 210 220

Ile Ala Asn Gln Asn Gly Ile Val Leu Asn Gly Val Lys Thr Ile Asn 225 230 235 240

Ala Asn Arg Phe Val Ala Thr Thr Ser Ser Thr Ile Asp Pro Glu Gln
245 250 255

Met Gln Leu Asn Val Thr Gln Gly Thr Val Thr Ile Gly Val Asp Gly 260 265 270

Phe Ala Thr Asp Gly Leu Pro Tyr Leu Asp Ile Ile Ala Lys Lys Ile 275 280 285

Glu Gln Lys Gln Ala Ile Thr Lys Glu Arg Thr Gly Asn Ser Glu Thr 290 295 300

- Asp Ile Thr Phe Val Ala Gly Asn Ser Lys Tyr Asp Leu Lys Thr His 305 310 315 320
- Ser Gly Ala Ser Thr Gly Ala Met Tyr Gly Lys Asn Ile Lys Leu Ile 340 345 350
- Val Thr Asp Lys Gly Ala Gly Val Lys His Asp Gly Ile Ile Leu Ser 355 360 365
- Glu Ala Asp Ile Gln Ile Glu Thr His Glu Gly Asp Val Glu Leu Gly 370 380
- Asn Thr Lys Asn Asn Gln Asn Glu Asn Tyr Ala Lys Ala His Ala Glu 385 390 395 400
- Gly Asn Phe Thr Val Lys Gly Gly Lys His Val Ile Ile Gly Lys Glu
  405 410 415
- Val Lys Ala Asn Lys Ala Val Asp Ile Gln Ala Gln Glu Thr Thr Val 420 425 430
- Arg Gln Asn Ala Lys Leu Thr Ala Lys Thr Ser Ala Lys Ile Thr Ala 435 440 445
- Ser Lys Ser Val Asn Leu Glu Asp Asn Ala Lys Leu Ile Ala Asn Glu 450 455 460
- Leu Ser Thr Thr Thr Asn Lys Leu Thr Asn Lys Gly Ser Ile Tyr Gly 465 470 475 480
- Lys Lys Val Thr Leu Asp Ala Asp Asn Leu Val Asn Ser Lys Glu Ile 485 490 495
- Tyr Ala Ser Ser Glu Leu Asp Ile Gln Thr Lys Gly Arg Asp Leu Leu 500 505 510
- Leu Glu Asp Gly Val Asn Gln Pro Leu Ser Phe Leu Lys Gly Ala Ser 515 520 525
- Leu Leu Ala Pro Gly Phe Val Asn Thr Gly Leu Ile His Ser Asn Gly 530 535 540
- Asn Ala Lys Leu Thr Phe Lys Asp Asp Thr Ser Phe Val Thr Glu Gly 545 550 555 560
- Asn Asn Phe Ile Thr Ala Lys Asp Asn Leu Glu Ile Thr Ala Lys Asn 565 570 575
- Val Gln Ile Asp Gln Ala Lys Asn Ile Gln Leu Asn Ala Asn Ile Thr 580 585 590
- Ile Asn Thr Lys Ser Gly Phe Val Asn Tyr Gly Thr Leu Ala Ser Ala 595 600 605
- Gln Asn Leu Thr Ile Asn Thr Glu Gln Gly Ser Ile Tyr Asn Ile Gly 610 615 620
- Gly Ile Leu Gly Ala Gly Lys Ser Leu Asn Leu Ser Ala Lys Arg Gly 625 630 635 640

Glu	Asn	Gln	Gly	Gly 645	Tyr	Leu	Ile	Asn	Gln 650	Gly	Lys	Ser	Leu	Leu 655	His	
Ser	Glu	Gly	Ala 660	Met	Asn	Leu	Thr	Ala 665	Asp	Arg	Thr	Val	Tyr 670	Asn	Leu	
Gly	Asn	Ile 675	Phe	Ala	Lys	Gly	Asp 680	Ala	Thr	Ile	Asn	Ala 685	Asn	Ala	Leu	
Ile	Asn 690	Asp	Val	Thr	Leu	Thr 695	Gly	Arg	Leu	Glu	Tyr 700	Gln	Asp	Leu	Lys	
Lys 705	Asp	Tyr	Thr	Arg	Tyr 710	Tyr	Arg	Ile	Asn	Glu 715	Thr	Ala	Lys	His	Gly 720	
Trp	His	Asn	Asn	Phe 725	Tyr	Glu	Leu	Asn	Val 730	Asp	Arg	Val	Ser			
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	L> CI	os 1)	(249)	)												
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gct Ala	cgt Arg	tta Leu	gta Val 20	gcc Ala	gaa Glu	aag Lys	ttc Phe	att Ile 25	aaa Lys	gcc Ala	caa Gln	tgt Cys	gta Val 30	gaa Glu	gca Ala	96
tta Leu	aca Thr	ttg Leu 35	gct Ala	ttg Leu	att Ile	gag Glu	ggt Gly 40	gtc Val	gag Glu	cac His	ttt Phe	gtg Val 45	ctg Leu	gaa Glu	ggt Gly	144
gag Glu	gag Glu 50	gaa Glu	agc Ser	aaa Lys	agg Arg	gga Gly 55	cat His	agt Ser	att Ile	aag Lys	gtt Val 60	vai	tta Leu	aaa Lys	gga Gly	192
agt Ser 65	cac His	gaa Glu	gtt Val	att Ile	aag Lys 70	tca Ser	gag Glu	gtg Val	aat Asn	aca Thr 75	Asn	gaa Glu	aaa Lys	aat Asn	cat His 80	240
_	aat Asn	cat His	ta													251
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Ala Arg Leu Val Ala Glu Lys Phe Ile Lys Ala Gln Cys Val Glu Ala
Leu Thr Leu Ala Leu Ile Glu Gly Val Glu His Phe Val Leu Glu Gly
Glu Glu Glu Ser Lys Arg Gly His Ser Ile Lys Val Val Leu Lys Gly
Ser His Glu Val Ile Lys Ser Glu Val Asn Thr Asn Glu Lys Asn His
Cys Asn His
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                                     10
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                                                                   96
Asn Lys Gly Ile Asn Gly Val Ser Gly Val Thr Gln Pro Leu Tyr Phe
                                 25
gac att aac gac age teg act gat gtg aac tat etc aat gaa caa gge
                                                                   144
Asp Ile Asn Asp Ser Ser Thr Asp Val Asn Tyr Leu Asn Glu Gln Gly
                             40
atc acg tgt tgc gtg aat cat aat ggc ttt cgt ttt tgg ggc tta cgc
                                                                   192
Ile Thr Cys Cys Val Asn His Asn Gly Phe Arg Phe Trp Gly Leu Arg
acg act gca gaa gat cca tta ttc aag ttt gaa gtg tac acc cgc act
                                                                   240
Thr Thr Ala Glu Asp Pro Leu Phe Lys Phe Glu Val Tyr Thr Arg Thr
 65
gca caa atc tta aaa gat acg att gca ggg gcg ttt gat tgg gca gtg
Ala Gln Ile Leu Lys Asp Thr Ile Ala Gly Ala Phe Asp Trp Ala Val
gat aaa gat att tot gto acg cta gtg aaa gat att att gaa gca atc
Asp Lys Asp Ile Ser Val Thr Leu Val Lys Asp Ile Ile Glu Ala Ile
            100
aat gcg aag tgg cgt gat tac acc aca aaa ggc tac tta att ggc ggt
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Asn Ala Lys Trp Arg Asp Tyr Thr Thr Lys Gly Tyr Leu Ile Gly Gly

115 120 125

aaa gcg tgg ctt aat aaa gag ctt aac agt gca acg aat tta aaa gat 432
Lys Ala Trp Leu Asn Lys Glu Leu Asn Ser Ala Thr Asn Leu Lys Asp
130

gcg aag ttg ttg atc tct tat gat tat cac cca gta cca ccg ctc gaa 480
Ala Lys Leu Leu Ile Ser Tyr Asp Tyr His Pro Val Pro Pro Leu Glu
145

cag cta ggc ttt aat cag tac att tct gat gaa tac ctt gtt gat ttt
Gln Leu Gly Phe Asn Gln Tyr Ile Ser Asp Glu Tyr Leu Val Asp Phe
165

tca aat cgt tta gca tcg ta
Ser Asn Arg Leu Ala Ser
180

<210> 121

<211> 182

<212> PRT

<213> Pasteurella multocida

<400> 121

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Asp Ile Asn Asp Ser Ser Thr Asp Val Asn Tyr Leu Asn Glu Gln Gly 35 40 45

Ile Thr Cys Cys Val Asn His Asn Gly Phe Arg Phe Trp Gly Leu Arg 50 55 60

Thr Thr Ala Glu Asp Pro Leu Phe Lys Phe Glu Val Tyr Thr Arg Thr 65 70 75 80

Ala Gln Ile Leu Lys Asp Thr Ile Ala Gly Ala Phe Asp Trp Ala Val 85 90 95

Asp Lys Asp Ile Ser Val Thr Leu Val Lys Asp Ile Ile Glu Ala Ile 100 105 110

Asn Ala Lys Trp Arg Asp Tyr Thr Thr Lys Gly Tyr Leu Ile Gly Gly
115 120 125

Lys Ala Trp Leu Asn Lys Glu Leu Asn Ser Ala Thr Asn Leu Lys Asp 130 135 140

Ala Lys Leu Leu Ile Ser Tyr Asp Tyr His Pro Val Pro Pro Leu Glu 145 150 155 160

Gln Leu Gly Phe Asn Gln Tyr Ile Ser Asp Glu Tyr Leu Val Asp Phe 165 170 175

Ser Asn Arg Leu Ala Ser 180

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<213> Actinobacillus pleuropneumoniae
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                                                                    69
Asp Lys Phe Lys Ile Leu Ser
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                                      10
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                                         10
ttg gca agc atg aca ta
                                                                    64
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Ala Ser Met Thr
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<220>
<223> apvB
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<221> CDS
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<400> 126
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Leu Ile Ser Phe Pro Phe Ile Thr Phe Ala Ser Asn Val Asn Gly Ala
                                                                   96
gaa att gga ttg gga gga gcc cgt gag agt agt att tac tat tct aaa
Glu Ile Gly Leu Gly Gly Ala Arg Glu Ser Ser Ile Tyr Tyr Ser Lys
cat aaa gta gca aca aat ccc ttt tta gca ctt gat ctt tct tta ggt
                                                                   144
His Lys Val Ala Thr Asn Pro Phe Leu Ala Leu Asp Leu Ser Leu Gly
aat ttt tat atg aga ggg act gca gga att agc gaa ata gga tat gaa
                                                                   192
Asn Phe Tyr Met Arg Gly Thr Ala Gly Ile Ser Glu Ile Gly Tyr Glu
     50
                         55
caa tot tto act gac aat tto ago gta toa ctg ttt gtt aac coa ttt
                                                                   240
Gln Ser Phe Thr Asp Asn Phe Ser Val Ser Leu Phe Val Asn Pro Phe
 65
gat ggt ttt tca att aaa gga aaa gac ttg tta cct gga tat caa agt
Asp Gly Phe Ser Ile Lys Gly Lys Asp Leu Leu Pro Gly Tyr Gln Ser
                                      90
att caa act cgc aaa act caa ttt gcc ttt ggt tgg gga tta aat tat
                                                                   336
Ile Gln Thr Arg Lys Thr Gln Phe Ala Phe Gly Trp Gly Leu Asn Tyr
aat ttg gga ggt tta ttc ggc tta aat gat act ttt ata tcc ttg gaa
                                                                   384
Asn Leu Gly Gly Leu Phe Gly Leu Asn Asp Thr Phe Ile Ser Leu Glu
gga aaa agc gga aaa cgt ggt gcg agt agt aat gtc agc tta ctt aaa
                                                                   432
Gly Lys Ser Gly Lys Arg Gly Ala Ser Ser Asn Val Ser Leu Leu Lys
tcg ttt aat atg acg aaa aat tgg aaa gtt tca cca tat att ggc tca
                                                                   480
Ser Phe Asn Met Thr Lys Asn Trp Lys Val Ser Pro Tyr Ile Gly Ser
                                         155
agt tat tat tca tct aaa tat aca gat tat tac ttt ggt att aaa caa
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Ser	Туг	ту1	Sei	Ser 165	Lys	Tyr	Thr	: Asp	Tyr 170		Phe	Gly	Ile	Lys 175	Gln	
tcc Ser	gaa Glu	ı tta ı Lei	a ggt a Gly 180	/ Asr	aaa Lys	att Ile	aca Thr	tcc Ser 185	Val	tat Tyr	aaa Lys	. cct Pro	aaa Lys 190	Ala	gct Ala	576
tat Tyr	gca Ala	aca Thr 195	His	ata Ile	ggt Gly	att Il∈	aat Asn 200	Thr	gat Asp	tat Tyr	gct Ala	ttc Phe 205	Thr	aac Asn	aat Asn	624
ctt Leu	ggc Gly 210	Met	ggt Gly	tta Leu	tct Ser	gto Val 215	Gly	tgg Trp	at							653
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	0> 1 Ile		Phe	Pro	Phe	Ile	Thr	Phe	Ala 10	Ser	Asn	Val	Asn	Gly 15	Ala	
Glu	Ile	Gly	Leu 20	Gly	Gly	Ala	Arg	Glu 25	Ser	Ser	Ile	Tyr	Tyr 30	Ser	Lys	
His	Lys	Val 35	Ala	Thr	Asn	Pro	Phe 40	Leu	Ala	Leu	Asp	Leu 45	Ser	Leu	Gly	
Asn	Phe 50	Tyr	Met	Arg	Gly	Thr 55	Ala	Gly	Ile	Ser	Glu 60	Ile	Gly	Tyr	Glu	
Gln 65	Ser	Phe	Thr	Asp	Asn 70	Phe	Ser	Val	Ser	Leu 75	Phe	Val	Asn	Pro	Phe 80	
Asp	Gly	Phe	Ser	Ile 85	Lys	Gly	Lys	Asp	Leu 90	Leu	Pro	Gly	Tyr	Gln 95	Ser	
Ile	Gln	Thr	Arg 100	Lys	Thr	Gln	Phe	Ala 105	Phe	Gly	Trp	Gly	Leu 110	Asn	Tyr	
Asn	Leu	Gly 115	Gly	Leu	Phe	Gly	Leu 120	Asn	Asp	Thr	Phe	Ile 125	Ser	Leu	Glu	
Gly	Lys 130	Ser	Gly	Lys	Arg	Gly 135	Ala	Ser	Ser	Asn	Val 140	Ser	Leu	Leu	Lys	
Ser 145	Phe	Asn	Met	Thr	Lys 150	Asn	Trp	Lys	Val	Ser 155	Pro	Tyr	Ile	Gly	Ser 160	
Ser	Tyr	Tyr	Ser	Ser 165	Lys	Tyr	Thr	Asp	Tyr 170	Tyr	Phe	Gly	Ile	Lys 175	Gln	
Ser	Glu	Leu	Gly 180	Asn	Lys	Ile	Thr	Ser 185	Val	Tyr	Lys	Pro	Lys 190	Ala	Ala	
Tyr	Ala	Thr 195	His	Ile	Gly	Ile	Asn 200	Thr	Asp	Tyr	Ala	Phe 205	Thr	Asn	Asn	
Leu	Gly	Met	Gly	Leu	Ser	Val	Gly	Trp								

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atg gct cgc cag att tta tca gcg gcg gag ttg ctc att gca aag gaa
                                                                    96
Met Ala Arg Gln Ile Leu Ser Ala Ala Glu Leu Leu Ile Ala Lys Glu
             20
ggt ttg caa aat tta tcg atg agg aaa atc gca agt gaa gcc ggt atc
                                                                    144
Gly Leu Gln Asn Leu Ser Met Arg Lys Ile Ala Ser Glu Ala Gly Ile
gca aca ggc acg ctt tat ctc tat ttc aaa acg aaa gac gag tta ctg
                                                                    192
Ala Thr Gly Thr Leu Tyr Leu Tyr Phe Lys Thr Lys Asp Glu Leu Leu
gat tgt ttg gcg gaa caa tta cat gaa cga tat tat cgt tat ctg aat
                                                                    240
Asp Cys Leu Ala Glu Gln Leu His Glu Arg Tyr Tyr Arg Tyr Leu Asn
                     70
                                          75
at
                                                                    242
<210> 129
<211> 80
<212> PRT
<213> Actinobacillus pleuropneumoniae
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Met Trp Arg Met Gly Asp Phe Met Ser Lys Lys Glu Arg Leu Asn Asp
Met Ala Arg Gln Ile Leu Ser Ala Ala Glu Leu Leu Ile Ala Lys Glu
Gly Leu Gln Asn Leu Ser Met Arg Lys Ile Ala Ser Glu Ala Gly Ile
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<210> 130

75

Ala Thr Gly Thr Leu Tyr Leu Tyr Phe Lys Thr Lys Asp Glu Leu Leu

Asp Cys Leu Ala Glu Gln Leu His Glu Arg Tyr Tyr Arg Tyr Leu Asn

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<220>
<221> CDS
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Asn Ile Gln Lys Thr Val Ile Ala Ser Gly Thr Leu Gln Ala Thr Glu
caa gta gat att ggt gca caa gta tct ggg cag att aag cat att tta
Gln Val Asp Ile Gly Ala Gln Val Ser Gly Gln Ile Lys His Ile Leu
gta caa gaa gga cag aag gtt aaa aaa ggt gag cta tta gct gta att
                                                                   144
Val Gln Glu Gly Gln Lys Val Lys Lys Gly Glu Leu Leu Ala Val Ile
gat cca cgt ctg gct gaa acg gaa tta aaa cta gca aaa gct gag cta
                                                                   192
Asp Pro Arg Leu Ala Glu Thr Glu Leu Lys Leu Ala Lys Ala Glu Leu
     50
                         55
gca aat gct tct gct aat ttg gat aca aaa aat aat ctt aag caa
                                                                   240
Ala Asn Ala Ser Ala Asn Leu Asp Thr Lys Lys Ile Asn Leu Lys Gln
ctg caa tca gat tgg gaa cgt cat caa cgt ttg ata cga acc aat gcg
                                                                   288
Leu Gln Ser Asp Trp Glu Arg His Gln Arg Leu Ile Arg Thr Asn Ala
aca agc caa aag gaa aca gaa gaa gca aaa agt aga tta aat acg gcc
                                                                   336
Thr Ser Gln Lys Glu Thr Glu Glu Ala Lys Ser Arg Leu Asn Thr Ala
                                105
aaa gca gaa ctt caa att gcg caa aat aat cta gat atc gct aaa atc
                                                                   384
Lys Ala Glu Leu Gln Ile Ala Gln Asn Asn Leu Asp Ile Ala Lys Ile
                            120
aga gtg gaa aaa gct gaa acc gaa cta gga tat aca gaa att cgt tct
                                                                   432
Arg Val Glu Lys Ala Glu Thr Glu Leu Gly Tyr Thr Glu Ile Arg Ser
                        135
                                            140
cca ctt gat gca aca gta att tca gta ttt gcg caa aat ggt caa act
                                                                   480
Pro Leu Asp Ala Thr Val Ile Ser Val Phe Ala Gln Asn Gly Gln Thr
tta gtc acc acc caa caa gta cca gtg ctg atg aaa tta gct aat at
                                                                   527
Leu Val Thr Thr Gln Gln Val Pro Val Leu Met Lys Leu Ala Asn
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<sup>&</sup>lt;210> 131

<sup>&</sup>lt;211> 175

<sup>&</sup>lt;212> PRT

<sup>&</sup>lt;213> Actinobacillus pleuropneumoniae

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Gln Val Asp Ile Gly Ala Gln Val Ser Gly Gln Ile Lys His Ile Leu
Val Gln Glu Gly Gln Lys Val Lys Lys Gly Glu Leu Leu Ala Val Ile
Asp Pro Arg Leu Ala Glu Thr Glu Leu Lys Leu Ala Lys Ala Glu Leu
Ala Asn Ala Ser Ala Asn Leu Asp Thr Lys Lys Ile Asn Leu Lys Gln
Leu Gln Ser Asp Trp Glu Arg His Gln Arg Leu Ile Arg Thr Asn Ala
Thr Ser Gln Lys Glu Thr Glu Glu Ala Lys Ser Arg Leu Asn Thr Ala
Lys Ala Glu Leu Gln Ile Ala Gln Asn Asn Leu Asp Ile Ala Lys Ile
                            120
Arg Val Glu Lys Ala Glu Thr Glu Leu Gly Tyr Thr Glu Ile Arg Ser
Pro Leu Asp Ala Thr Val Ile Ser Val Phe Ala Gln Asn Gly Gln Thr
Leu Val Thr Thr Gln Gln Val Pro Val Leu Met Lys Leu Ala Asn
<210> 132
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                                                                   48
Met Ala Gly Ala Lys Glu Ile Arg Thr Lys Ile Ala Ser Val Lys Asn
act caa aaa atc acc aaa gca atg gaa atg gtt gct acc tct aaa atg
                                                                   96
Thr Gln Lys Ile Thr Lys Ala Met Glu Met Val Ala Thr Ser Lys Met
cgt aaa acg caa gag cgt atg gct gcc agt cgt cct tat tcg gaa aca
Arg Lys Thr Gln Glu Arg Met Ala Ala Ser Arg Pro Tyr Ser Glu Thr
atc cgt aag gtg att agc cat att qcq aaa qqa aqc att qqt tat aaq
Ile Arg Lys Val Ile Ser His Ile Ala Lys Gly Ser Ile Gly Tyr Lys
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50 55 60

														ctt Leu		240
														tta Leu 95		288
														gtt Val		336
gtt Val	gag Glu	ctt Leu 115	ggt Gly	tta Leu	gta Val	ggg ggg	tcg Ser 120	aaa Lys	ggc Gly	gta Val	agc Ser	ttt Phe 125	tac Tyr	caa Gln	aat Asn	384
														aat Asn		432
														gcg Ala		480
														ttt Phe 175		528
														tta Leu		576
														tat Tyr		624
tat Tyr	gaa Glu 210	ccg Pro	aat Asn	cca Pro	caa Gln	gtt Val 215	tta Leu	ttg Leu	gat Asp	agt Ser	tta Leu 220	ctt Leu	gtt Val	cgt Arg	tat Tyr	672
tta Leu 225	gaa Glu	act Thr	cag Gln	gta Val	tac Tyr 230	caa Gln	gca Ala	gtt Val	gta Val	gat Asp 235	aac Asn	cta Leu	gct Ala	tct Ser	gaa Glu 240	720
caa Gln	gcc Ala	gct Ala	cga Arg	atg Met 245	gta Val	gcg Ala	atg Met	aaa Lys	gcc Ala 250	gca Ala	aca Thr	gat Asp	aat Asn	gcg Ala 255	ggt Gly	768
														caa Gln		816
agc Ser	att Ile	aca Thr 275	aat Asn	gaa Glu	tta Leu	aac Asn	gaa Glu 280	att Ile	gtt Val	gcg Ala	ggt Gly	gcc Ala 285	gca Ala	gca Ala	att Ile	864
taa																867

<210> 133

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<213> Actinobacillus pleuropneumoniae

<400> 133

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Arg Lys Thr Gln Glu Arg Met Ala Ala Ser Arg Pro Tyr Ser Glu Thr
35 40 45

Ile Arg Lys Val Ile Ser His Ile Ala Lys Gly Ser Ile Gly Tyr Lys
50 55 60

His Pro Phe Leu Thr Glu Arg Asp Ile Lys Lys Val Gly Tyr Leu Val 65 70 75 80

Val Ser Thr Asp Arg Gly Leu Cys Gly Gly Leu Asn Ile Asn Leu Phe 85 90 95

Lys Ala Thr Leu Asn Glu Phe Lys Thr Trp Lys Asp Lys Asp Val Ser

Val Glu Leu Gly Leu Val Gly Ser Lys Gly Val Ser Phe Tyr Gln Asn 115 120 125

Leu Gly Leu Asn Val Arg Ser Gln Val Thr Gly Leu Gly Asp Asn Pro 130 135 140

Glu Met Glu Arg Ile Val Gly Ala Val Asn Glu Met Ile Asn Ala Phe 145 150 155 160

Arg Asn Gly Glu Val Asp Ala Val Tyr Val Ala Tyr Asn Arg Phe Glu 165 170 175

Asn Thr Met Ser Gln Lys Pro Val Ile Ala Gln Leu Leu Pro Leu Pro 180 185 190

Lys Leu Asp Asp Asp Glu Leu Asp Thr Lys Gly Ser Trp Asp Tyr Ile 195 200 205

Tyr Glu Pro Asn Pro Gln Val Leu Leu Asp Ser Leu Leu Val Arg Tyr 210 215 220

Leu Glu Thr Gln Val Tyr Gln Ala Val Val Asp Asn Leu Ala Ser Glu 225 230 235 240

Gln Ala Ala Arg Met Val Ala Met Lys Ala Ala Thr Asp Asn Ala Gly
245 250 255

Thr Leu Ile Asp Glu Leu Gln Leu Val Tyr Asn Lys Ala Arg Gln Ala 260 265 270

Ser Ile Thr Asn Glu Leu Asn Glu Ile Val Ala Gly Ala Ala Ile 275 280 285

<210> 134

<211> 534

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		tpH														
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					caa Gln											96
					gtt Val											144
					agc Ser											192
_		_			gat Asp 70			~~~					_	_	_	240
					ctg Leu											288
					gaa Glu											336
_	_		_		agt Ser		_		_	_			_			384
					ggt Gly											432
					ggc Gly 150											480
					ggt Gly											528
ttg Leu	taa															534
<211	)> 13 -> 17 !> PF	77														

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Asp Phe	Ala	Leu 20	Glu	Gln	Gly	Gln	Leu 25	Asp	Lys	Trp	Gln	Glu 30	Met	Leu	
Gln Phe	Ser 35		Phe	Val	Ala	Glu 40	Asn	Glu	Gln	Val	Ala 45	Glu	Tyr	Ile	
Asn Ser 50	Ser	Leu	Ala	Ser	Gly 55	Gln	Ile	Ser	Glu	Thr 60	Phe	Ile	Lys	Ile	
Cys Gly 65	Asp	Gln	Leu	Asp 70	Gln	Tyr	Gly	Gln	Asn 75	Phe	Ile	Arg	Val	Met 80	
Ala Glu	Asn	Lys	Arg 85	Leu	Ala	Val	Leu	Pro 90	Met	Val	Phe	Asp	Thr 95	Phe	
Val Ser	Leu	Arg 100	Ala	Glu	His	Glu	Ala 105	Val	Lys	Asp	Val	Thr 110	Ile	Val	
Ser Ala	Asn 115	Glu	Leu	Ser	Gln	Ala 120	Gln	Glu	Asp	Lys	Ile 125	Ala	Lys	Ala	
Met Glu 130	Lys	Arg	Leu	Gly	Gln 135	Lys	Val	Arg	Leu	Thr 140	Asn	Gln	Ile	Asp	
Asn Ser 145	Leu	Ile	Ala	Gly 150	Val	Ile	Ile	Lys	Tyr 155	Asp	Asp	Val	Val	Ile 160	
Asp Gly	Ser	Ser	Arg 165	Gly	Gln	Leu	Asn	Arg 170	Leu	Ala	Ser	Ala	Leu 175	Ser	
Leu															
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atg cag Met Gln	gaa Glu	gaa Glu 20	gtc Val	gct Ala	aat Asn	ttc Phe	gcc Ala 25	gat Asp	cct Pro	gcg Ala	gac Asp	cgc Arg 30	gcc Ala	act Thr	96

cag gaa gaa gtc agt ctt gaa tta aga aac cgt gac cgt gag cgt

Gln	Glu	Glu 35	Glu	Phe	Ser	Leu	Glu 40	Leu	Arg	Asn	Arg	Asp 45	Arg	Glu	Arg	
	ttg Leu 50															192
	tac Tyr															240
	gaa Glu		_	_			~	_	_		_	_				288
	gaa Glu									taa						321
<211 <212	)> 13 L> 10 2> PF 3> Ac	)6 RT	baci	llus	s ple	europ	oneur	nonia	ae							
	)> 13 Trp		Val	Gln 5	Ile	Met	Asp	Glu	Ala 10	Glu	Arg	Thr	Lys	Asn 15	Gln	
Met	Gln	Glu	Glu 20	Val	Ala	Asn	Phe	Ala 25	Asp	Pro	Ala	Asp	Arg 30	Ala	Thr	
Gln	Glu	Glu 35	Glu	Phe	Ser	Leu	Glu 40	Leu	Arg	Asn	Arg	Asp 45	Arg	Glu	Arg	
Lys	Leu 50	Leu	Lys	Lys	Ile	Glu 55	Gln	Thr	Leu	Asn	Ser 60	Ile	Ala	Glu	Asp	
Glu 65	Tyr	Gly	Tyr	Cys	Glu 70	Thr	Cys	Gly	Val	Glu 75	Ile	Gly	Leu	Arg	Arg 80	
Leu	Glu	Ala	Arg	Pro 85	Thr	Ala	Asp	Met	Cys 90	Ile	Asp	Cys	Lys	Thr 95	Leu	
Ala	Glu	Ile	Arg 100	Glu	Lys	Gln	Met	Gly 105	Leu							
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<220 <223	> > dn	aK														
	> > CD > (1		30)													
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Ala Glu Phe Glu Glu Val Lys Asp Asn Lys
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Ala Glu Phe Glu Glu Val Lys Asp Asn Lys
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Met Glu Gln Met Leu Glu Leu Gln Gly His Val Asp Tyr Ile Ile
96
Leu Gly Leu Leu Leu Met Ser Val Val Leu Val Trp Lys Ile Ile
                                25
gaa cgc gta ctt ttc tac aaa caa ttg gat gtg acc aaa tat gac acg
                                                                144
Glu Arg Val Leu Phe Tyr Lys Gln Leu Asp Val Thr Lys Tyr Asp Thr
                            40
cta caa gat ttg gaa att gat acc act cgc aat tta acc acc att tcc
Leu Gln Asp Leu Glu Ile Asp Thr Thr Arg Asn Leu Thr Thr Ile Ser
    50
act atc ggt gcc aac gcc cct tat atc ggt tta tta gga acc gta tta
                                                                240
Thr Ile Gly Ala Asn Ala Pro Tyr Ile Gly Leu Leu Gly Thr Val Leu
                                                                288
ggg atc tta ctt acc ttc tat cat tta ggg cat tcc ggc ggt gat att
Gly Ile Leu Leu Thr Phe Tyr His Leu Gly His Ser Gly Gly Asp Ile
                                                                336
gac gcc gca tcc att atg gtt cac ctt tcg ctt gca tta aaa gca acc
Asp Ala Ala Ser Ile Met Val His Leu Ser Leu Ala Leu Lys Ala Thr
           100
gca gcc ggt atc tta gtc gct att ccg gca atg atg ttc tac agc ggt
                                                                384
Ala Ala Gly Ile Leu Val Ala Ile Pro Ala Met Met Phe Tyr Ser Gly
       115
                           120
ttt aac cgt aaa gtg gat gaa agc aaa ctt aaa tgg caa gcg att caa
Phe Asn Arg Lys Val Asp Glu Ser Lys Leu Lys Trp Gln Ala Ile Gln
   130
                       135
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gct cgt aaa gcc aat caa taa
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 Ala Arg Lys Ala Asn Gln
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 Leu Gly Leu Leu Leu Met Ser Val Val Leu Val Trp Lys Ile Ile
 Glu Arg Val Leu Phe Tyr Lys Gln Leu Asp Val Thr Lys Tyr Asp Thr
 Leu Gln Asp Leu Glu Ile Asp Thr Thr Arg Asn Leu Thr Thr Ile Ser
 Thr Ile Gly Ala Asn Ala Pro Tyr Ile Gly Leu Leu Gly Thr Val Leu
Gly Ile Leu Leu Thr Phe Tyr His Leu Gly His Ser Gly Gly Asp Ile
Asp Ala Ala Ser Ile Met Val His Leu Ser Leu Ala Leu Lys Ala Thr
Ala Ala Gly Ile Leu Val Ala Ile Pro Ala Met Met Phe Tyr Ser Gly
                             120
Phe Asn Arg Lys Val Asp Glu Ser Lys Leu Lys Trp Gln Ala Ile Gln
Ala Arg Lys Ala Asn Gln
<210> 142
<211> 720
<212> DNA
<213> Actinobacillus pleuropneumoniae
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<220>
<221> CDS
<222> (1)..(717)
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Met Leu Lys Asn Lys Leu Ser Val Leu Ala Ile Val Ala Gly Thr Phe
                                     10
gtt toa got caa act goa ttt goa gog gat caa aaa tto att gac gat
                                                                   96
Val Ser Ala Gln Thr Ala Phe Ala Ala Asp Gln Lys Phe Ile Asp Asp
             20
                                 25
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tca Ser	tca Ser	tat Tyr 35	gca Ala	gtc Val	ggc	gta Val	ttg Leu 40	atg Met	ggt Gly	aaa Lys	aat Asn	atc Ile 45	gaa Glu	ggc Gly	gtc Val	144
gtt Val	gaa Glu 50	Ser	caa Gln	aaa Lys	gaa Glu	att Ile 55	ttt Phe	tct Ser	tat Tyr	aac Asn	caa Gln 60	gat Asp	aaa Lys	atc Ile	ttg Leu	192
gcg Ala 65	ggt Gly	gtc Val	caa Gln	gat Asp	acc Thr 70	Ile	aaa Lys	aaa Lys	acc Thr	ggt Gly 75	aaa Lys	tta Leu	acc Thr	gat Asp	gaa Glu 80	240
gat Asp	cta Leu	caa Gln	aaa Lys	caa Gln 85	tta Leu	aaa Lys	tcg Ser	ctt Leu	gat Asp 90	act Thr	tat Tyr	ctt Leu	gca Ala	agt Ser 95	caa Gln	288
gaa Glu	agc Ser	aaa Lys	att Ile 100	gcg Ala	gcg Ala	gag Glu	aaa Lys	agc Ser 105	aaa Lys	gca Ala	acc Thr	gta Val	gaa Glu 110	gcc Ala	ggt Gly	336
			cgt Arg													384
gct Ala	tcc Ser 130	ggt Gly	tta Leu	ctt Leu	tat Tyr	aaa Lys 135	att Ile	gaa Glu	aaa Lys	gcc Ala	ggc Gly 140	acg Thr	ggc Gly	gaa Glu	tcg Ser	432
cct Pro 145	aaa Lys	gcg Ala	gaa Glu	gat Asp	acc Thr 150	gtt Val	aaa Lys	gtt Val	cac His	tat Tyr 155	aaa Lys	Gly 999	aca Thr	tta Leu	acc Thr 160	480
gat Asp	ggt Gly	acg Thr	gta Val	ttc Phe 165	gat Asp	agc Ser	tca Ser	tac Tyr	gat Asp 170	cgc Arg	ggt Gly	gag Glu	ccg Pro	att Ile 175	gaa Glu	528
ttc Phe	caa Gln	tta Leu	aac Asn 180	caa Gln	tta Leu	att Ile	ccg Pro	ggt Gly 185	tgg Trp	att Ile	gaa Glu	gcg Ala	att Ile 190	cca Pro	atg Met	576
ttg Leu	aaa Lys	aaa Lys 195	gly ggc	gga Gly	aaa Lys	atg Met	gaa Glu 200	atc Ile	gtc Val	gtt Val	ccg Pro	cct Pro 205	gaa Glu	ctt Leu	ggt Gly	624
tac Tyr	ggc Gly 210	gaa Glu	cgc Arg	caa Gln	gca Ala	ggt Gly 215	aag Lys	att Ile	ccg Pro	gca Ala	agt Ser 220	tca Ser	acc Thr	tta Leu	aaa Lys	672
ttc Phe 225	gag Glu	att Ile	gaa Glu	ttg Leu	tta Leu 230	gat Asp	ttc Phe	aaa Lys	gcg Ala	gcc Ala 235	gaa Glu	gcg Ala	aaa Lys	aaa Lys	taa	720
<210 <211 <212 <213	> 23 > PR	9 T	baci	llus	ple	urop	neum	onia	.e							
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Val Ser Ala Gln Thr Ala Phe Ala Ala Asp Gln Lys Phe Ile Asp Asp Ser Ser Tyr Ala Val Gly Val Leu Met Gly Lys Asn Ile Glu Gly Val Val Glu Ser Gln Lys Glu Ile Phe Ser Tyr Asn Gln Asp Lys Ile Leu Ala Gly Val Gln Asp Thr Ile Lys Lys Thr Gly Lys Leu Thr Asp Glu Asp Leu Gln Lys Gln Leu Lys Ser Leu Asp Thr Tyr Leu Ala Ser Gln Glu Ser Lys Ile Ala Ala Glu Lys Ser Lys Ala Thr Val Glu Ala Gly Asn Lys Phe Arg Thr Asp Tyr Glu Lys Gln Ser Gly Val Lys Lys Thr 120 Ala Ser Gly Leu Leu Tyr Lys Ile Glu Lys Ala Gly Thr Gly Glu Ser Pro Lys Ala Glu Asp Thr Val Lys Val His Tyr Lys Gly Thr Leu Thr Asp Gly Thr Val Phe Asp Ser Ser Tyr Asp Arg Gly Glu Pro Ile Glu Phe Gln Leu Asn Gln Leu Ile Pro Gly Trp Ile Glu Ala Ile Pro Met Leu Lys Lys Gly Gly Lys Met Glu Ile Val Val Pro Pro Glu Leu Gly 200 Tyr Gly Glu Arg Gln Ala Gly Lys Ile Pro Ala Ser Ser Thr Leu Lys Phe Glu Ile Glu Leu Leu Asp Phe Lys Ala Ala Glu Ala Lys Lys 225 230 <210> 144 <211> 290 <212> DNA <213> Actinobacillus pleuropneumoniae <220> <223> HI0379 <220> <221> CDS <222> (3)..(287) <400> 144 tg cat agc gtg aga ggt ccg ggc ggt tat caa ctc ggt aag caa His Ser Val Arg Gly Pro Gly Gly Gly Tyr Gln Leu Gly Lys Gln cct gaa gag att agt gtg ggg atg att att gcg gcg gtg aat gaa aat Pro Glu Glu Ile Ser Val Gly Met Ile Ile Ala Ala Val Asn Glu Asn

ctc gac gta acc aaa tgt aaa ggt agc ggc aac tgt agc aaa aac tct 143 Leu Asp Val Thr Lys Cys Lys Gly Ser Gly Asn Cys Ser Lys Asn Ser 191 cag tgc tta acc cat cat tta tgg gaa cgt tta gaa gaa caa atc ggt Gln Cys Leu Thr His His Leu Trp Glu Arg Leu Glu Glu Gln Ile Gly 55 239

gtg ttt tta aat acg att act tta gcg gaa ctt gtt gaa gaa cat tcg Val Phe Leu Asn Thr Ile Thr Leu Ala Glu Leu Val Glu Glu His Ser

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290 taa

<210> 145

<211> 95

<212> PRT

<213> Actinobacillus pleuropneumoniae

<400> 145

His Ser Val Arg Gly Pro Gly Gly Gly Tyr Gln Leu Gly Lys Gln Pro

Glu Glu Ile Ser Val Gly Met Ile Ile Ala Ala Val Asn Glu Asn Leu

Asp Val Thr Lys Cys Lys Gly Ser Gly Asn Cys Ser Lys Asn Ser Gln

Cys Leu Thr His His Leu Trp Glu Arg Leu Glu Glu Gln Ile Gly Val 50 55

Phe Leu Asn Thr Ile Thr Leu Ala Glu Leu Val Glu Glu His Ser Asp

His Asp Cys Glu Lys Glu His Cys His Asp His Ser His Lys His

<210> 146

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<213> Actinobacillus pleuropneumoniae

<220>

<223> hupA

<220>

<221> CDS

<222> (1)..(270)

<400> 146

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							gac Asp 40									144
							aat Asn									192
							gca Ala									240
			_				tta Leu			taa						273
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Ser	Lys	Lys	Asp 20	Ala	Lys	Ala	Ala	Leu 25	Glu	Ala	Thr	Leu	Asn 30	Ala	Ile	
Ser	Glu	Ser 35	Leu	Lys	Asn	Gly	Asp 40	Thr	Val	Gln	Leu	Ile 45	Gly	Phe	Gly	
Thr	Phe 50	Lys	Val	Asn	Glu	Arg 55	Asn	Ala	Arg	Thr	Gly 60	Arg	Asn	Pro	Arg	
Thr 65	Gly	Glu	Glu	Ile	Lys 70	Ile	Ala	Ala	Ser	Lys 75	Val	Pro	Ala	Phe	Val 80	
Ala	Gly	Lys	Ala	Leu 85	Lys	Asp	Leu	Val	Lys 90							
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	L> CI	os L)	(549)	)												
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gta Val	att Ile	gtc Val 35	gaa Glu	cgt Arg	tat Tyr	tca Ser	act Thr 40	ttg Leu	ggc Gly	ggt Gly	gta Val	tgc Cys 45	tta Leu	aac Asn	gta Val	144
ggt Gly	tgt Cys 50	att Ile	ccg Pro	tct Ser	aaa Lys	gca Ala 55	tta Leu	tta Leu	cac His	gtt Val	gca Ala 60	aaa Lys	gtt Val	atc Ile	gaa Glu	192
gaa Glu 65	gca Ala	aaa Lys	cac His	gca Ala	gag Glu 70	aaa Lys	aac Asn	ggt Gly	att Ile	act Thr 75	ttc Phe	ggt Gly	gag Glu	ccc Pro	aac Asn 80	240
att Ile	gat Asp	tta Leu	gat Asp	aaa Lys 85	gtg Val	cgt Arg	gcg Ala	ggt Gly	aaa Lys 90	gaa Glu	gcg Ala	gtt Val	gtt Val	tct Ser 95	aaa Lys	288
tta Leu	acc Thr	ggc Gly	ggt Gly 100	tta Leu	gcg Ala	ggt Gly	atg Met	gct Ala 105	aaa Lys	gca Ala	cgt Arg	aaa Lys	gta Val 110	aca Thr	gta Val	336
gtg Val	gaa Glu	ggt Gly 115	tta Leu	gcg Ala	gcg Ala	ttt Phe	acc Thr 120	gat Asp	ccg Pro	aat Asn	act Thr	tta Leu 125	gta Val	gct Ala	cgt Arg	384
gac Asp	cgt Arg 130	gac Asp	ggt Gly	aat Asn	ccg Pro	aca Thr 135	acg Thr	att Ile	aaa Lys	ttt Phe	gat Asp 140	tat Tyr	gca Ala	att Ile	att Ile	432
gca Ala 145	gcc Ala	ggt Gly	tct Ser	cgt Arg	ccg Pro 150	att Ile	cag Gln	ctt Leu	ccg Pro	ttc Phe 155	att Ile	cca Pro	cac His	gaa Glu	gat Asp 160	480
ccg Pro	cgt Arg	gtg Val	tgg Trp	gat Asp 165	tct Ser	acg Thr	gat Asp	gca Ala	ctt Leu 170	aaa Lys	tta Leu	aaa Lys	gaa Glu	gta Val 175	ccc Pro	528
	Lys	att Ile	Thr				cc									551

<210> 149

<211> 183

<212> PRT

<213> Actinobacillus pleuropneumoniae

<400> 149

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Ala Gly Tyr Ser Ala Ala Phe Arg Cys Ala Asp Leu Gly Leu Glu Thr
20 25 30

Val Ile Val Glu Arg Tyr Ser Thr Leu Gly Gly Val Cys Leu Asn Val 35 40 45

Gly Cys Ile Pro Ser Lys Ala Leu Leu His Val Ala Lys Val Ile Glu 50 55 60

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Glu Ala Lys His Ala Glu Lys Asn Gly Ile Thr Phe Gly Glu Pro Asn
 65
Ile Asp Leu Asp Lys Val Arg Ala Gly Lys Glu Ala Val Val Ser Lys
Leu Thr Gly Gly Leu Ala Gly Met Ala Lys Ala Arg Lys Val Thr Val
Val Glu Gly Leu Ala Ala Phe Thr Asp Pro Asn Thr Leu Val Ala Arg
Asp Arg Asp Gly Asn Pro Thr Thr Ile Lys Phe Asp Tyr Ala Ile Ile
                        135
Ala Ala Gly Ser Arg Pro Ile Gln Leu Pro Phe Ile Pro His Glu Asp
                    150
Pro Arg Val Trp Asp Ser Thr Asp Ala Leu Lys Leu Lys Glu Val Pro
Glu Lys Ile Thr His Tyr Gly
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Met Lys Lys Ser Leu Val Ala Leu Thr Val Leu Ser Ala Ala Val
                                     10
                                                                   96
get caa gea geg eea caa caa aat aet tte tae gea ggt geg aaa gea
Ala Gln Ala Ala Pro Gln Gln Asn Thr Phe Tyr Ala Gly Ala Lys Ala
ggt tgg gcg tca ttc cat gat ggt atc gaa caa tta gat tca gct aaa
                                                                   144
Gly Trp Ala Ser Phe His Asp Gly Ile Glu Gln Leu Asp Ser Ala Lys
aac aca gat cgc ggt aca aaa tac ggt atc aac cgt aat tca gta act
                                                                   192
Asn Thr Asp Arg Gly Thr Lys Tyr Gly Ile Asn Arg Asn Ser Val Thr
                         55
tac ggc gta ttc ggc ggt tac caa att tta aac caa gac aaa tta ggt
                                                                   240
Tyr Gly Val Phe Gly Gly Tyr Gln Ile Leu Asn Gln Asp Lys Leu Gly
tta gcg gct gaa tta ggt tat gac tat ttc ggt cgt gtg cgc ggt tct
Leu Ala Ala Glu Leu Gly Tyr Asp Tyr Phe Gly Arg Val Arg Gly Ser
                 85
                                     90
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	a aaa u Lys			Gly												336
	c ggt s Gly															384
tt Le	a gac u Asp 130	Val	tac Tyr	ggt Gly	aaa Lys	gta Val 135	ggt Gly	atc Ile	gca Ala	tta Leu	gta Val 140	aac Asn	aat Asn	aca Thr	tat Tyr	432
aa Ly 14	a aca s Thr 5	ttc Phe	aat Asn	gca Ala	gca Ala 150	caa Gln	gag Glu	aaa Lys	gtg Val	aaa Lys 155	act Thr	cgt Arg	cgt Arg	ttc Phe	caa Gln 160	480
ag Se	t tct r Ser	tta Leu	att Ile	tta Leu 165	ggt Gly	gcg Ala	ggt Gly	gtt Val	gag Glu 170	tac Tyr	gca Ala	att Ile	ctt Leu	cct Pro 175	gaa Glu	528
tt. Le	a gcg u Ala	gca Ala	cgt Arg 180	gtt Val	gaa Glu	tac Tyr	caa Gln	tgg Trp 185	tta Leu	aac Asn	aac Asn	gca Ala	ggt Gly 190	aaa Lys	gca Ala	576
	c tac r Tyr															624
	c agt e Ser 210	Ser														672
gc: Al: 22!	a ccg a Pro	gtt Val	gca Ala	gct Ala	ccg Pro 230	gca Ala	gtt Val	gaa Glu	act Thr	aaa Lys 235	aac Asn	ttc Phe	gca Ala	ttc Phe	agc Ser 240	720
	gac r Asp															768
gca Ala	a aca a Thr	gca Ala	tta Leu 260	gat Asp	gca Ala	atg Met	caa Gln	acc Thr 265	gaa Glu	atc Ile	aat Asn	aac Asn	gca Ala 270	ggt Gly	tta Leu	816
tca Sei	a aat : Asn	gct Ala 275	gcg Ala	atc Ile	caa Gln	gta Val	aac Asn 280	ggt Gly	tac Tyr	acg Thr	gac Asp	cgt Arg 285	atc Ile	ggt Gly	aaa Lys	864
gaa Glu	a gct 1 Ala 290	tca Ser	aac Asn	tta Leu	aaa Lys	ctt Leu 295	tca Ser	caa Gln	cgt Arg	cgt Arg	gcg Ala 300	gaa Glu	aca Thr	gta Val	gct Ala	912
aac Asr 305	tac Tyr	atc Ile	gtt Val	tct Ser	aaa Lys 310	ggt Gly	gct Ala	ccg Pro	gca Ala	gct Ala 315	aac Asn	gta Val	act Thr	gca Ala	gta Val 320	960
ggt Gl <sub>y</sub>	tac Tyr	ggt Gly	gaa Glu	gca Ala 325	aac Asn	cct Pro	gta Val	acc Thr	ggc Gly 330	gca Ala	aca Thr	tgt Cys	gac Asp	aaa Lys 335	gtt Val	1008
aaa Lys	ggt Gly	cgt Arg	aaa Lys	gca Ala	tta Leu	atc Ile	gct Ala	tgc Cys	tta Leu	gca Ala	ccg Pro	gat Asp	cgt Arg	cgt Arg	gtt Val	1056

340 345 350

gaa gtt caa gtt caa ggt act aaa gaa gta act atg taa Glu Val Gln Val Gln Gly Thr Lys Glu Val Thr Met 355 360 1095

<210> 151

<211> 364

<212> PRT

<213> Actinobacillus pleuropneumoniae

<400> 151

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Gly Trp Ala Ser Phe His Asp Gly Ile Glu Gln Leu Asp Ser Ala Lys 35 40 45

Asn Thr Asp Arg Gly Thr Lys Tyr Gly Ile Asn Arg Asn Ser Val Thr 50 55 60

Tyr Gly Val Phe Gly Gly Tyr Gln Ile Leu Asn Gln Asp Lys Leu Gly 65 70 75 80

Leu Ala Ala Glu Leu Gly Tyr Asp Tyr Phe Gly Arg Val Arg Gly Ser 85 90 95

Glu Lys Pro Asn Gly Lys Ala Asp Lys Lys Thr Phe Arg His Ala Ala 100 105 110

His Gly Ala Thr Ile Ala Leu Lys Pro Ser Tyr Glu Val Leu Pro Asp 115 120 125

Leu Asp Val Tyr Gly Lys Val Gly Ile Ala Leu Val Asn Asn Thr Tyr 130 140

Lys Thr Phe Asn Ala Ala Gln Glu Lys Val Lys Thr Arg Arg Phe Gln 145 150 155 160

Ser Ser Leu Ile Leu Gly Ala Gly Val Glu Tyr Ala Ile Leu Pro Glu 165 170 175

Leu Ala Ala Arg Val Glu Tyr Gln Trp Leu Asn Asn Ala Gly Lys Ala 180 185

Ser Tyr Ser Thr Leu Asn Arg Met Gly Ala Thr Asp Tyr Arg Ser Asp 195 200 205

Ile Ser Ser Val Ser Ala Gly Leu Ser Tyr Arg Phe Gly Gln Gly Ala 210 215 220

Ala Pro Val Ala Ala Pro Ala Val Glu Thr Lys Asn Phe Ala Phe Ser 225 230 235 240

Ser Asp Val Leu Phe Ala Phe Gly Lys Ser Asn Leu Lys Pro Ala Ala 245 250 255

Ala Thr Ala Leu Asp Ala Met Gln Thr Glu Ile Asn Asn Ala Gly Leu

260 265 270

Ser Asn Ala Ala Ile Gln Val Asn Gly Tyr Thr Asp Arg Ile Gly Lys 275 280 285

Glu Ala Ser Asn Leu Lys Leu Ser Gln Arg Arg Ala Glu Thr Val Ala 290 295 300

Asn Tyr Ile Val Ser Lys Gly Ala Pro Ala Ala Asn Val Thr Ala Val 305 310 315 320

Gly Tyr Gly Glu Ala Asn Pro Val Thr Gly Ala Thr Cys Asp Lys Val 325 330 335

Lys Gly Arg Lys Ala Leu Ile Ala Cys Leu Ala Pro Asp Arg Val 340 345 350

Glu Val Gln Val Gln Gly Thr Lys Glu Val Thr Met

<210> 152

<211> 1110

<212> DNA

<213> Actinobacillus pleuropneumoniae

<220>

<223> Omp5

<220>

<221> CDS

<222> (1)..(1107)

<400> 152

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Met Lys Lys Ser Leu Val Ala Leu Ala Val Leu Ser Ala Ala Ala Val
1 5 10 15

gct caa gca gct cca caa caa aat act ttc tac gca ggt gcg aaa gtt 96 Ala Gln Ala Ala Pro Gln Gln Asn Thr Phe Tyr Ala Gly Ala Lys Val 20 25 30

ggt caa tca tct cac cac ggt gtt aac caa tta aaa tct ggt cac 144 Gly Gln Ser Ser Phe His His Gly Val Asn Gln Leu Lys Ser Gly His 35 40 45

gat gat cgt tat aat gat aaa aca cgt aag tat ggt atc aac cgt aac 192 Asp Asp Arg Tyr Asn Asp Lys Thr Arg Lys Tyr Gly Ile Asn Arg Asn
50
60

tct gta act tac ggt gta ttc ggc ggt tac caa atc tta aac caa aat 240 Ser Val Thr Tyr Gly Val Phe Gly Gly Tyr Gln Ile Leu Asn Gln Asn 65 70 75 80

aac ttc ggt tta gca gct gaa tta ggc tat gac tac tac ggt cgc gta 288 Asn Phe Gly Leu Ala Ala Glu Leu Gly Tyr Asp Tyr Tyr Gly Arg Val

cgt ggt aac gta gat gaa ttc cgt aca gtt aaa cac tct gct cac ggt 336 Arg Gly Asn Val Asp Glu Phe Arg Thr Val Lys His Ser Ala His Gly 100 105 110

				aaa Lys				_	-			_		_	384
_				ggt Gly			_	_	_		_				432
	 	_		act Thr 150		_									480
				tta Leu											528
				gtt Val											576
		-		gtt Val	_					_		_			624
				cac His											672
				gca Ala 230											720
				tca Ser											768
		_	_	gca Ala		_		_	_	_			_		816
				gca Ala											864
				gaa Glu											912
				aac Asn 310			_							-	960
				ggt Gly											1008
				aaa Lys											1056
				gaa Glu											1104

360

365

atg taa Met

1110

<210> 153

<211> 369

<212> PRT

<213> Actinobacillus pleuropneumoniae

<400> 153

12 : 🗒

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þ.á 1.1

1.2

12 : # : #

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:1

Met Lys Lys Ser Leu Val Ala Leu Ala Val Leu Ser Ala Ala Val

Ala Gln Ala Ala Pro Gln Gln Asn Thr Phe Tyr Ala Gly Ala Lys Val

Gly Gln Ser Ser Phe His His Gly Val Asn Gln Leu Lys Ser Gly His

Asp Asp Arg Tyr Asn Asp Lys Thr Arg Lys Tyr Gly Ile Asn Arg Asn

Ser Val Thr Tyr Gly Val Phe Gly Gly Tyr Gln Ile Leu Asn Gln Asn

Asn Phe Gly Leu Ala Ala Glu Leu Gly Tyr Asp Tyr Tyr Gly Arg Val

Arg Gly Asn Val Asp Glu Phe Arg Thr Val Lys His Ser Ala His Gly 105

Leu Asn Leu Ala Leu Lys Pro Ser Tyr Glu Val Leu Pro Asp Leu Asp

Val Tyr Gly Lys Val Gly Ile Ala Val Val Arg Asn Asp Tyr Lys Lys

Tyr Gly Ala Glu Asn Thr Asn Glu Ser Thr Thr Lys Phe His Lys Leu 150

Lys Ala Ser Thr Ile Leu Gly Ala Gly Val Glu Tyr Ala Ile Leu Pro

Glu Leu Ala Ala Arg Val Glu Tyr Gln Tyr Leu Asn Lys Ala Gly Asn 185

Leu Asn Lys Ala Leu Val Arg Ser Gly Thr Gln Asp Val Asp Phe Gln 200

Tyr Ala Pro Asp Ile His Ser Val Thr Ala Gly Leu Ser Tyr Arg Phe 215

Gly Gln Gly Ala Val Ala Pro Val Val Glu Pro Glu Val Val Thr Lys

Asn Phe Ala Phe Ser Ser Asp Val Leu Phe Asp Phe Gly Lys Ser Ser

Leu Lys Pro Ala Ala Ala Thr Ala Leu Asp Ala Ala Asn Thr Glu Ile 260 265

Ala	Asn	Leu 275		Leu	Ala	Thr	280		ılle	Gln	Val	Asn 285		Tyr	Thr	
Asp	Arg 290		e Gly	Lys	Glu	Ala 295		Asn	ı Leu	. Lys	Leu 300		Gln	Arg	Arg	
Ala 305		Thr	Val	Ala	Asn 310		Leu	. Val	Ser	Lys 315		Gln	Asn	Pro	Ala 320	
Asn	. Val	Thr	Ala	. Val 325		Tyr	Gly	Glu	Ala 330		Pro	Val	Thr	Gly 335	Ala	
Thr	Cys	Asp	Ala 340		Lys	Gly	Arg	Lys 345		Leu	Ile	Ala	Cys 350	Leu	Ala	
Pro	Asp	Arg 355		Val	Glu	Val	Gln 360		Gln	Gly	Ala	Lys 365	Asn	Val	Ala	
Met																
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<22 <22		np n	ew													
	1> C		(107	4)												
	0> 1! att		gaa	ttc	gta	aaa	gaa	gcq	ggt	aaa	ccq	cat	taa	qat	tga	48
Asn 1	Ile	Lys	Glu	Phe 5	Val	Lys	Glu	Ala	Gly 10	Lys	Pro	Arg	Trp	Asp 15	Trp	
gtt Val	gcg Ala	ccg Pro	gaa Glu 20	ccg Pro	aat Asn	acc Thr	gca Ala	tta Leu 25	atc Ile	aac Asn	caa Gln	gtt Val	aaa Lys 30	gcg Ala	tta Leu	96
gcg Ala	gaa Glu	gcg Ala 35	cgt Arg	atc Ile	ggc Gly	gat Asp	gcg Ala 40	tat Tyr	cgt Arg	att Ile	aca Thr	gaa Glu 45	aaa Lys	caa Gln	gcg Ala	144
cgt Arg	tac Tyr 50	gaa Glu	caa Gln	atc Ile	gat Asp	gca Ala 55	att Ile	aaa Lys	gcg Ala	gat Asp	gtt Val 60	atc Ile	gca Ala	caa Gln	tta Leu	192
acc Thr 65	gca Ala	caa Gln	gac Asp	gaa Glu	acc Thr 70	gtt Val	tct Ser	gaa Glu	ggt Gly	gcg Ala 75	att Ile	att Ile	gat Asp	att Ile	att Ile 80	240
acc Thr	gca Ala	tta Leu	gaa Glu	agt Ser 85	tct Ser	att Ile	gtt Val	cgc Arg	ggt Gly 90	cgt Arg	att Ile	att Ile	gcc Ala	ggc Gly 95	gaa Glu	288
ccg Pro	cgt Arg	att Ile	gac Asp 100	ggt Gly	cgt Arg	acg Thr	gta Val	gat Asp 105	acg Thr	gtt Val	cgt Arg	gca Ala	tta Leu 110	gac Asp	att Ile	336

					cct Pro											384
					tta Leu											432
					gaa Glu 150											480
					cct Pro											528
					cgt Arg											576
	_			_	atg Met	_		_	_	_		_		_		624
					att Ile											672
					tct Ser 230											720
					ggt Gly											768
					tca Ser											816
Asp	Met	Asp 275	Phe	Lys	gta Val	Āla	Gly 280	Thr	Arg	Glu	Gly	Val 285	Thr	Āla	Leu	864
caa Gln	atg Met 290	gat Asp	att Ile	aaa Lys	atc Ile	gaa Glu 295	ggt Gly	atc Ile	acg Thr	cct Pro	gaa Glu 300	att Ile	atg Met	caa Gln	atc Ile	912
					aaa Lys 310											960
gaa Glu	caa Gln	gcg Ala	att Ile	cct Pro 325	gca Ala	cct Pro	cgt Arg	gcc Ala	gat Asp 330	att Ile	tcc Ser	gat Asp	ttt Phe	gcg Ala 335	cct Pro	1008
cgt Arg	att Ile	cat His	acg Thr 340	atg Met	aag Lys	atc Ile	gat Asp	ccg Pro 345	aag Lys	aaa Lys	atc Ile	aaa Lys	gac Asp 350	gtg Val	atc Ile	1056
				gcg Ala	gtt Val	at										1076

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<211> 358

<212> PRT

<213> Actinobacillus pleuropneumoniae

<400> 155

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Val Ala Pro Glu Pro Asn Thr Ala Leu Ile Asn Gln Val Lys Ala Leu 20 25 30

Ala Glu Ala Arg Ile Gly Asp Ala Tyr Arg Ile Thr Glu Lys Gln Ala
35 40 45

Arg Tyr Glu Gln Ile Asp Ala Ile Lys Ala Asp Val Ile Ala Gln Leu 50 60

Thr Ala Gln Asp Glu Thr Val Ser Glu Gly Ala Ile Ile Asp Ile Ile 65 70 75 80

Thr Ala Leu Glu Ser Ser Ile Val Arg Gly Arg Ile Ile Ala Gly Glu 85 90 95

Pro Arg Ile Asp Gly Arg Thr Val Asp Thr Val Arg Ala Leu Asp Ile
100 105 110

Cys Thr Gly Val Leu Pro Arg Thr His Gly Ser Ala Ile Phe Thr Arg 115 120 125

Gly Glu Thr Gln Ala Leu Ala Val Ala Thr Leu Gly Thr Glu Arg Asp 130 135 140

Ala Gln Ile Val Asp Glu Leu Thr Gly Glu Lys Ser Asp Arg Phe Leu 145 150 155 160

Phe His Tyr Asn Phe Pro Pro Tyr Ser Val Gly Glu Thr Gly Arg Ile 165 170 175

Gly Ser Pro Lys Arg Arg Glu Ile Gly His Gly Arg Leu Ala Lys Arg 180 185 190

Gly Val Leu Ala Val Met Pro Thr Ala Glu Glu Phe Pro Tyr Val Val 195 200 205

Arg Val Val Ser Glu Ile Thr Glu Ser Asn Gly Ser Ser Ser Met Ala 210 215 220

Ser Val Cys Gly Ala Ser Leu Ala Leu Met Asp Ala Gly Val Pro Ile 225 230 235 240

Lys Ala Ala Val Ala Gly Ile Ala Met Gly Leu Val Lys Glu Glu Glu 245 250 255

Lys Phe Val Val Leu Ser Asp Ile Leu Gly Asp Glu Asp His Leu Gly 260 265 270

Asp Met Asp Phe Lys Val Ala Gly Thr Arg Glu Gly Val Thr Ala Leu 275 280 285

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Gln Met Asp Ile Lys Ile Glu Gly Ile Thr Pro Glu Ile Met Gln Ile
Ala Leu Asn Gln Ala Lys Gly Ala Arg Met His Ile Leu Ser Val Met
                                         315
Glu Gln Ala Ile Pro Ala Pro Arg Ala Asp Ile Ser Asp Phe Ala Pro
                325
Arg Ile His Thr Met Lys Ile Asp Pro Lys Lys Ile Lys Asp Val Ile
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Gly Lys Gly Gly Ala Val
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<210> 156
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<222> (1)..(1053)
<400> 156
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tta aca gcg tgt aat gaa gaa aag cca aaa gcg gct gaa gca gcg gct
                                                                   96
Leu Thr Ala Cys Asn Glu Glu Lys Pro Lys Ala Ala Glu Ala Ala Ala
caa ccg gca gca gcg gga aca gtt cac ctt tat act tgg act gaa tat
Gln Pro Ala Ala Gly Thr Val His Leu Tyr Thr Trp Thr Glu Tyr
         35
gtg cct gaa ggc ttg tta gat gaa ttt aca aag caa acc ggt atc aaa
Val Pro Glu Gly Leu Leu Asp Glu Phe Thr Lys Gln Thr Gly Ile Lys
     50
                         55
gta gag gtt tca agc ctt gaa tct aac gaa acc atg tat gcg aaa tta
                                                                   240
Val Glu Val Ser Ser Leu Glu Ser Asn Glu Thr Met Tyr Ala Lys Leu
 65
                     70
                                          75
aaa tta caa ggt aaa gac ggc ggt tac gat gtt atc gca cct tct aac
                                                                   288
Lys Leu Gln Gly Lys Asp Gly Gly Tyr Asp Val Ile Ala Pro Ser Asn
tac ttc gtt tca aaa atg gcg aaa gaa ggt atg tta gcg gaa tta gat
                                                                   336
Tyr Phe Val Ser Lys Met Ala Lys Glu Gly Met Leu Ala Glu Leu Asp
cac gca aaa ctt cct gta atc aaa gag tta aac caa gat tgg tta aac
                                                                   384
His Ala Lys Leu Pro Val Ile Lys Glu Leu Asn Gln Asp Trp Leu Asn
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aaa cct tat gac caa ggt aac aaa tac tct tta ccg caa tta tta ggt

Lys	Pro 130	Tyr	Asp	Gln	Gly	Asn 135	Lys	Tyr	Ser	Leu	Pro 140	Gln	Leu	Leu	Gly	
gca Ala 145	ccg Pro	ggt Gly	atc Ile	gca Ala	ttt Phe 150	aac Asn	tca Ser	aat Asn	gac Asp	tat Tyr 155	aag Lys	ggc	gat Asp	gcg Ala	ttc Phe 160	480
act Thr	tct Ser	tgg Trp	ggt Gly	gat Asp 165	tta Leu	tgg Trp	aaa Lys	cct Pro	gag Glu 170	ttt Phe	gcg Ala	aat Asn	aaa Lys	gta Val 175	caa Gln	528
tta Leu	tta Leu	gat Asp	gac Asp 180	gca Ala	cgt Arg	gaa Glu	gta Val	ttt Phe 185	aac Asn	att Ile	gcg Ala	tta Leu	tta Leu 190	aaa Lys	tta Leu	576
ggt Gly	aaa Lys	aac Asn 195	cct Pro	aat Asn	aca Thr	acc Thr	aat Asn 200	ccg Pro	gaa Glu	gag Glu	att Ile	aaa Lys 205	gcg Ala	gct Ala	tac Tyr	624
gaa Glu	gag Glu 210	tta Leu	aga Arg	aaa Lys	tta Leu	cgt Arg 215	cca Pro	aac Asn	gta Val	ctt Leu	tct Ser 220	ttc Phe	act Thr	tca Ser	gac Asp	672
aac Asn 225	cca Pro	gcg Ala	aac Asn	tca Ser	ttt Phe 230	atc Ile	gca Ala	ggt Gly	gaa Glu	gta Val 235	tct Ser	gta Val	ggt Gly	caa Gln	tta Leu 240	720
tgg Trp	aac Asn	ggt Gly	tct Ser	gta Val 245	cgt Arg	att Ile	gcg Ala	aaa Lys	aaa Lys 250	gaa Glu	caa Gln	gcg Ala	ccg Pro	gta Val 255	aac Asn	768
atg Met	gtg Val	ttc Phe	cca Pro 260	aaa Lys	gaa Glu	ggt Gly	cct Pro	gta Val 265	ctt Leu	tgg Trp	gtt Val	gat Asp	acg Thr 270	tta Leu	gcc Ala	816
att Ile	ccg Pro	gcg Ala 275	aat Asn	gcg Ala	aaa Lys	aac Asn	aaa Lys 280	gaa Glu	aat Asn	gcg Ala	cat His	aag Lys 285	tta Leu	atc Ile	aac Asn	864
tac Tyr	tta Leu 290	tta Leu	agc Ser	gca Ala	ccg Pro	gtt Val 295	gcg Ala	gaa Glu	aaa Lys	tta Leu	acg Thr 300	tta Leu	gaa Glu	atc Ile	ggt Gly	912
tat Tyr 305	ccg Pro	act Thr	tca Ser	aac Asn	gta Val 310	gaa Glu	gcg Ala	tta Leu	aaa Lys	aca Thr 315	tta Leu	cca Pro	aaa Lys	gag Glu	att Ile 320	960
acc Thr	gaa Glu	gat Asp	ccg Pro	gca Ala 325	atc Ile	tat Tyr	ccg Pro	aca Thr	gct Ala 330	gat Asp	gtg Val	tta Leu	aaa Lys	gcg Ala 335	gca Ala	1008
caa Gln	tgg Trp	caa Gln	gac Asp 340	gat Asp	gta Val	ggt Gly	aat Asn	gca Ala 345	atc Ile	gaa Glu	ctt Leu	tac Tyr	gaa Glu 350	aaa Lys	ta	1055

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<sup>&</sup>lt;212> PRT

<sup>&</sup>lt;213> Actinobacillus pleuropneumoniae

<sup>&</sup>lt;400> 157

Met Lys Lys Leu Ala Gly Leu Phe Ala Ala Gly Leu Ala Thr Val Ala Leu Thr Ala Cys Asn Glu Glu Lys Pro Lys Ala Ala Glu Ala Ala Ala Gln Pro Ala Ala Gly Thr Val His Leu Tyr Thr Trp Thr Glu Tyr Val Pro Glu Gly Leu Leu Asp Glu Phe Thr Lys Gln Thr Gly Ile Lys Val Glu Val Ser Ser Leu Glu Ser Asn Glu Thr Met Tyr Ala Lys Leu Lys Leu Gln Gly Lys Asp Gly Gly Tyr Asp Val Ile Ala Pro Ser Asn Tyr Phe Val Ser Lys Met Ala Lys Glu Gly Met Leu Ala Glu Leu Asp His Ala Lys Leu Pro Val Ile Lys Glu Leu Asn Gln Asp Trp Leu Asn 120 Lys Pro Tyr Asp Gln Gly Asn Lys Tyr Ser Leu Pro Gln Leu Leu Gly 135 Ala Pro Gly Ile Ala Phe Asn Ser Asn Asp Tyr Lys Gly Asp Ala Phe Thr Ser Trp Gly Asp Leu Trp Lys Pro Glu Phe Ala Asn Lys Val Gln 170 Leu Leu Asp Asp Ala Arg Glu Val Phe Asn Ile Ala Leu Leu Lys Leu Gly Lys Asn Pro Asn Thr Thr Asn Pro Glu Glu Ile Lys Ala Ala Tyr Glu Glu Leu Arg Lys Leu Arg Pro Asn Val Leu Ser Phe Thr Ser Asp Asn Pro Ala Asn Ser Phe Ile Ala Gly Glu Val Ser Val Gly Gln Leu Trp Asn Gly Ser Val Arg Ile Ala Lys Lys Glu Gln Ala Pro Val Asn Met Val Phe Pro Lys Glu Gly Pro Val Leu Trp Val Asp Thr Leu Ala

Ile Pro Ala Asn Ala Lys Asn Lys Glu Asn Ala His Lys Leu Ile Asn 275 280 285

260

Tyr Leu Leu Ser Ala Pro Val Ala Glu Lys Leu Thr Leu Glu Ile Gly
290 295 300

Tyr Pro Thr Ser Asn Val Glu Ala Leu Lys Thr Leu Pro Lys Glu Ile 305 310 315 320

Thr Glu Asp Pro Ala Ile Tyr Pro Thr Ala Asp Val Leu Lys Ala Ala 325 330 335

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cag cgt cga atg gat tac gaa ggc tac atc tca cgt agt ctg ctt aat
                                                                    96
Gln Arg Arg Met Asp Tyr Glu Gly Tyr Ile Ser Arg Ser Leu Leu Asn
cgt ttg ggt gaa tct gtg agc aat gtg cta agc gat gca caa gtt act
                                                                   144
Arg Leu Gly Glu Ser Val Ser Asn Val Leu Ser Asp Ala Gln Val Thr
                             40
ctc tcg tta tat atc gat ccg caa cgc tta acc gtt att aaa ggt acg
                                                                   192
Leu Ser Leu Tyr Ile Asp Pro Gln Arg Leu Thr Val Ile Lys Gly Thr
gcg aca gtg gaa gtg gaa ttc gat tgc caa cga tgc ggt aac ccg ttt
                                                                   240
Ala Thr Val Glu Val Glu Phe Asp Cys Gln Arg Cys Gly Asn Pro Phe
 65
aca caa acg ctt gac tgt tcg ttt tgt ttc agt ccg gtg tcc aat atg
Thr Gln Thr Leu Asp Cys Ser Phe Cys Phe Ser Pro Val Ser Asn Met
gat cag gcg gac aat ttg ccc gaa att tat gaa cca atc gaa gta aac
Asp Gln Ala Asp Asn Leu Pro Glu Ile Tyr Glu Pro Ile Glu Val Asn
gag ttc ggt gaa gta aat tta cta gat atg atc gaa gat gga ttt atc
                                                                   384
Glu Phe Gly Glu Val Asn Leu Leu Asp Met Ile Glu Asp Gly Phe Ile
        115
                            120
atc gaa ttg cct cta gtc ccg atg cat agt gaa gaa cac tgt gaa gtg
                                                                   432
Ile Glu Leu Pro Leu Val Pro Met His Ser Glu Glu His Cys Glu Val
                        135
tcc gtg agt gaa cag gtg ttt ggc gaa ttg cct gaa gaa ttg gcg aaa
                                                                   480
Ser Val Ser Glu Gln Val Phe Gly Glu Leu Pro Glu Glu Leu Ala Lys
                    150
aaa cct aac ccg ttc gct gta tta gct aat tta aag aaa aac tag
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Lys Pro Asn Pro Phe Ala Val Leu Ala Asn Leu Lys Lys Asn
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Gln Trp Gln Asp Asp Val Gly Asn Ala Ile Glu Leu Tyr Glu Lys

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Arg Leu Gly Glu Ser Val Ser Asn Val Leu Ser Asp Ala Gln Val Thr
Leu Ser Leu Tyr Ile Asp Pro Gln Arg Leu Thr Val Ile Lys Gly Thr
Ala Thr Val Glu Val Glu Phe Asp Cys Gln Arg Cys Gly Asn Pro Phe
Thr Gln Thr Leu Asp Cys Ser Phe Cys Phe Ser Pro Val Ser Asn Met
                 85
Asp Gln Ala Asp Asn Leu Pro Glu Ile Tyr Glu Pro Ile Glu Val Asn
Glu Phe Gly Glu Val Asn Leu Leu Asp Met Ile Glu Asp Gly Phe Ile
        115
Ile Glu Leu Pro Leu Val Pro Met His Ser Glu Glu His Cys Glu Val
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                                             140
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att acc gta gct gct gat aaa atc gaa gcg gct tac aaa gag caa tta
Ile Thr Val Ala Ala Asp Lys Ile Glu Ala Ala Tyr Lys Glu Gln Leu
aaa ggc tat gcg aaa aac gct cgt gta gac ggt ttc cgt aaa ggt aaa
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Lys	Gly	Tyr 35		Lys	Asn	Ala	Arg 40		Asp	Gly	Phe	Arg	_	Gly	Lys	
gta Val	ccg Pro 50	His	gca Ala	att Ile	ato	gaa Glu 55	Gln	cgt Arg	ttc Phe	ggt Gly	tta Leu 60	Ala	gct Ala	cgc Arg	caa Gln	192
gac Asp 65	Val	tta Leu	tcc Ser	gat Asp	gaa Glu 70	atg Met	caa Gln	cgt Arg	gcg Ala	ttc Phe 75	Phe	gat Asp	gcg Ala	gta Val	atc Ile 80	240
gct Ala	gag Glu	aaa Lys	att Ile	aac Asn 85	ctt Leu	gcc Ala	ggt Gly	cgt Arg	cct Pro 90	Thr	ttc Phe	aca Thr	ccg Pro	aac Asn 95	Asn	288
tac Tyr	caa Gln	ccg Pro	agt Ser 100	caa Gln	gaa Glu	ttc Phe	agc Ser	ttc Phe 105	act Thr	gca Ala	act Thr	ttt Phe	gaa Glu 110	gta Val	ttc Phe	336
ccg Pro	gaa Glu	gtt Val 115	gaa Glu	tta Leu	aaa Lys	ggc	tta Leu 120	gaa Glu	aat Asn	atc Ile	gaa Glu	gtt Val 125	gaa Glu	aaa Lys	ccg Pro	384
gtt Val	gta Val 130	gaa Glu	atc Ile	aca Thr	gaa Glu	gct Ala 135	gat Asp	tta Leu	gac Asp	aaa Lys	atg Met 140	atc Ile	gat Asp	gtg Val	tta Leu	432
Arg 145	Lys	caa Gln	Gln	Ala	Thr 150	Trp	Ala	Glu	Ser	Gln 155	Ala	Ala	Ala	Gln	Ala 160	480
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cgt Arg	atg Met	atc Ile 195	cct Pro	ggt Gly	ttt Phe	gaa Glu	gaa Glu 200	ggt Gly	atc Ile	gtt Val	ggt Gly	cac His 205	aaa Lys	gcc Ala	ggc Gly	624
Glu	Gln 210	ttc Phe	Asp	Ile	Asp	Val 215	Thr	Phe	Pro	Glu	Glu 220	Tyr	His	Ala	Glu	672
aac Asn 225	tta Leu	aaa Lys	ggt Gly	aaa Lys	gcg Ala 230	gcg Ala	aaa Lys	ttc Phe	gca Ala	att Ile 235	aca Thr	ctt Leu	aag Lys	aaa Lys	gta Val 240	720
gaa Glu	aat Asn	atc Ile	gta Val	tta Leu 245	cct Pro	gaa Glu	tta Leu	acc Thr	gaa Glu 250	gaa Glu	ttc Phe	gtg Val	aaa Lys	aaa Lys 255	ttc Phe	768
ggt Gly	tca Ser	Ala	aaa Lys 260	act Thr	gta Val	gaa Glu	gat Asp	tta Leu 265	cgt Arg	gcg Ala	gaa Glu	att Ile	aag Lys 270	aaa Lys	aat Asn	816
atg Met	Gln	cgt Arg 275	gaa Glu	ctt Leu	aaa Lys	aac Asn	gca Ala 280	gta Val	acc Thr	gca Ala	cgc Arg	gtt Val 285	aaa Lys	aac Asn	caa Gln	864

						-										
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gcg Ala 305	gta Val	gcg Ala	gaa Glu	gaa Glu	gtg Val 310	gac Asp	gta Val	tta Leu	cgt Arg	cgt Arg 315	caa Gln	gcg Ala	gtt Val	caa Gln	cgt Arg 320	960
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gaa Glu	gcg Ala	gat Asp	gca Ala 340	aaa Lys	cgt Arg	cgt Arg	gtt Val	caa Gln 345	gta Val	ggt Gly	tta Leu	tta Leu	ctt Leu 350	tca Ser	acc Thr	1056
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acg Thr	att Ile 370	gca Ala	gaa Glu	atc Ile	gct Ala	tca Ser 375	gct Ala	tac Tyr	gaa Glu	caa Gln	ccg Pro 380	gcg Ala	gaa Glu	gtt Val	gtt Val	1152
gct Ala 385	cat His	tat Tyr	gcg Ala	aaa Lys	aac Asn 390	cgt Arg	caa Gln	tta Leu	acc Thr	gaa Glu 395	aat Asn	atc Ile	cgt Arg	aac Asn	gta Val 400	1200
gtg Val	tta Leu	gaa Glu	gag Glu	caa Gln 405	gcg Ala	gtt Val	gaa Glu	gtt Val	gta Val 410	ctt Leu	gcg Ala	aaa Lys	gca Ala	aaa Lys 415	gta Val	1248
act Thr	gaa Glu	aaa Lys	gcg Ala 420	act Thr	tct Ser	ttt Phe	gat Asp	gaa Glu 425	gta Val	atg Met	gct Ala	caa Gln	caa Gln 430	gct Ala	caa Gln	1296
ggc Gly	taa															1302
<21 <21	0 > 10 1 > 4 2 > Pl 3 > A	33 RT	obac:	illu	s pl	euro	pneu	moni	ae							
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т1 о	Thr	7727	7.7.5	Δla	λαη	Lare	Tle	Glu	Δla	Δla	Tvr	Lvs	Glu	Gln	Leu	

Ile Thr Val Ala Ala Asp Lys Ile Glu Ala Ala Tyr Lys Glu Gln Leu 20 25 30

Lys Gly Tyr Ala Lys Asn Ala Arg Val Asp Gly Phe Arg Lys Gly Lys 35 40 45

Val Pro His Ala Ile Ile Glu Gln Arg Phe Gly Leu Ala Ala Arg Gln 50 55 60

Asp Val Leu Ser Asp Glu Met Gln Arg Ala Phe Phe Asp Ala Val Ile 65 70 75 80

Ala Glu Lys Ile Asn Leu Ala Gly Arg Pro Thr Phe Thr Pro Asn Asn

THE BOTH RESERVED AND SOUTH AND SOUT

Tyr	Gln	Pro	Ser 100	Gln	Glu	Phe	Ser	Phe 105	Thr	Ala	Thr	Phe	Glu 110	Val	Phe
Pro	Glu	Val 115	Glu	Leu	Lys	Gly	Leu 120	Glu	Asn	Ile	Glu	Val 125	Glu	Lys	Pro
Val	Val 130	Glu	Ile	Thr	Glu	Ala 135	Asp	Leu	Asp	Lys	Met 140	Ile	Asp	Val	Leu
Arg 145	Lys	Gln	Gln	Ala	Thr 150	Trp	Ala	Glu	Ser	Gln 155	Ala	Ala	Ala	Gln	Ala 160
Glu	Asp	Arg	Val	Val 165	Ile	Asp	Phe	Val	Gly 170	Ser	Val	Asp	Gly	Glu 175	Glu
Phe	Glu	Gly	Gly 180	Lys	Ala	Thr	Asp	Phe 185	Thr	Leu	Ala	Met	Gly 190	Gln	Ser
Arg	Met	Ile 195	Pro	Gly	Phe	Glu	Glu 200	Gly	Ile	Val	Gly	His 205	Lys	Ala	Gly
Glu	Gln 210	Phe	Asp	Ile	Asp	Val 215	Thr	Phe	Pro	Glu	Glu 220	Tyr	His	Ala	Glu
Asn 225	Leu	Lys	Gly	Lys	Ala 230	Ala	Lys	Phe	Ala	Ile 235	Thr	Leu	Lys	Lys	Val 240
Glu	Asn	Ile	Val	Leu 245	Pro	Glu	Leu	Thr	Glu 250	Glu	Phe	Val	Lys	Lys 255	Phe
Gly	Ser	Ala	Lys 260	Thr	Val	Glu	Asp	Leu 265	Arg	Ala	Glu	Ile	Lys 270	Lys	Asn
Met	Gln	Arg 27.5	Glu	Leu	Lys	Asn	Ala 280	Val	Thr	Ala	Arg	Val 285	Lys	Asn	Gln
Val	Ile 290	Asn	Gly	Leu	Ile	Ala 295	Gln	Asn	Glu	Ile	Glu 300	Val	Pro	Ala	Ala
Ala 305	Val	Ala	Glu	Glu	Val 310	Asp	Val	Leu	Arg	Arg 315	Gln	Ala	Val	Gln	Arg 320
Phe	Gly	Gly	Lys	Pro 325	Glu	Met	Ala	Ala	Gln 330	Leu	Pro	Ala	Glu	Leu 335	Phe
Glu	Ala	Asp	Ala 340	Lys	Arg	Arg	Val	Gln 345	Val	Gly	Leu	Leu	Leu 350	Ser	Thr
Val	Ile	Gly 355	Thr	Asn	Glu	Leu	Lys 360	Val	Asp	Glu	Lys	Arg 365	Val	Glu	Glu
Thr	Ile 370	Ala	Glu	Ile	Ala	Ser 375	Ala	Tyr	Glu	Gln	Pro 380	Ala	Glu	Val	Val
Ala 385	His	Tyr	Ala	Lys	Asn 390	Arg	Gln	Leu	Thr	Glu 395	Asn	Ile	Arg	Asn	Val 400
Val	Leu	Glu	Glu	Gln 405	Ala	Val	Glu	Val	Val 410	Leu	Ala	Lys	Ala	Lys 415	Val

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tttaaagatg acttttgttg tctgaattgt tctttaaaaa attggaaaca agctgaaaac 180
tgagagattt tcgaaagaaa gtctgagtag taaaagataa gtaattatct tgaaaatctt 240
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ctt gaa acg ctc tat atg ggc ttt gcg gcg act tta ctt gct gtg gta
                                                                   96
Leu Glu Thr Leu Tyr Met Gly Phe Ala Ala Thr Leu Leu Ala Val Val
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20 25 30

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												gtg Val				192
												gtg Val				240
												acg Thr				288
												cgt Arg				336
												gcg Ala 125				384
atg Met	ggc Gly 130	gca Ala	acg Thr	aat Asn	tgg Trp	caa Gln 135	gtg Val	gtc Val	agt Ser	aaa Lys	ttt Phe 140	tat Tyr	tta Leu	ccg Pro	gaa Glu	432
												tta Leu				480
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		_		_			_		_		_	gtc Val		_		576
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Leu Glu Thr Leu Tyr Met Gly Phe Ala Ala Thr Leu Leu Ala Val Val 20 25 30

Val Gly Leu Pro Ile Gly Phe Leu Ala Phe Leu Thr Gly Lys Gly Glu

- Ile Leu Glu Asn Pro Arg Leu His Gln Val Leu Asp Val Ile Ile Asn 50 55 60
- Ile Gly Arg Ser Val Pro Phe Ile Ile Leu Leu Val Val Leu Leu Pro 65 70 75 80
- Phe Thr Arg Leu Leu Val Gly Thr Thr Leu Gly Thr Thr Ala Ala Ile 85 90 95
- Val Pro Leu Ser Val Ser Ala Ile Pro Phe Phe Ala Arg Leu Thr Ser 100 105 110
- Asn Ala Leu Leu Glu Ile Pro Ala Gly Leu Thr Glu Ala Ala Lys Ser 115 120 125
- Met Gly Ala Thr Asn Trp Gln Val Val Ser Lys Phe Tyr Leu Pro Glu 130 135 140
- Ser Leu Pro Ile Leu Ile Asn Gly Ile Thr Leu Thr Leu Val Ala Leu 145 150 155 160
- Ile Gly Tyr Ser Ala Met Ala Gly Ala Val Gly Gly Gly Leu Gly 165 170 175
- Asn Leu Ala Ile Ser Tyr Gly Glu His Arg Asn Met Val Tyr Val Lys 180 185 190
- Trp Ile Ser Thr Ile Ile Ile Val Ala Ile Val Met Ile Ser Gln
  195 200 205